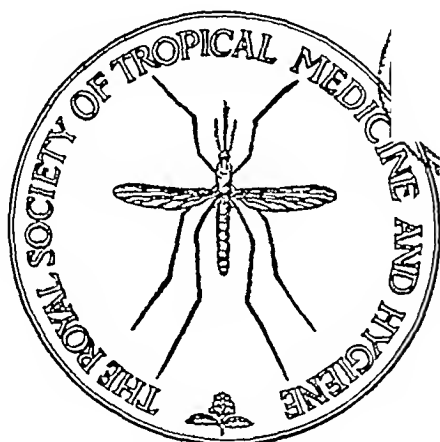


TRANSACTIONS
OF THE
ROYAL SOCIETY OF TROPICAL
MEDICINE AND HYGIENE.

PATRON - HIS MAJESTY THE KING



ZONAE TORRIDAE TUTAMEN

VOL. 42 1948-1949.

London

ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE,
MANSON HOUSE 26, PORTLAND PLACE, LONDON W 1

Telephone LANGHAM 2127

Telegrams ANOPHELES, LONDON

CONTENTS

Vol. 42, 1945-49.

No 1 Issued 7th July 1945.

LABORATORY MEETING 18th March, 1945.		PAGE
DEMONSTRATIONS		1
ORDINARY MEETING 18th April, 1945.		
PAPER Diseases of tropical origin in captive wild animals. R. E. Howell		17
DISCUSSION The President; Mr H. G. Martin; The President; Mr Harold Seed; The President; Professor Edward Hinde; Dr J. Uagar; Dr C. A. Hearn; Dr C. M. Worsley The President Dr Carmichael Low; Mr-Odr T C Morton; Dr R. E. Howell (in reply)		26
COMMUNICATIONS		
W. tern, J. M., Abdel Azim, M., and Hahawani, A. Investigations on the antibacterial action of miconid D (Nidochin)		37
Asted, L., Harding, R. D., and Gordon, M. A clinical and serological follow-up of yaws cases treated by acetylarsen and bismuth sodium potassium tartrate		45
Oliverhead, R. H. Anaemia and malaria in Indian troops on active service		60
McCarthy D D and Bagster Wilson D Dengue in the East African continent. Incidence in relation to <i>Aedes</i> prevalence and some clinical features		83
Giles, Alfred J. The tolerance of the merozoite and flagellate forms of <i>Leishmania chagasi</i> Fontana to variations of humidity and salinity		89
Brooker, Otto Thomas, Thomas, Esther E., and Brooker, Barbara. <i>Capillaria hepatica</i>		93
Yoffe, M. Non-pigmented malarial parasites in the bone marrow from mixed infection of <i>Leishmania</i> and <i>Plasmodium vivax</i>		99
Earle, K. Victor. Injuries produced by tropical water-beetles		101

CONTENTS.

No 2. Issued 17th September 1945.

ANNUAL GENERAL MEETING 17th June, 1945.		PAGE
BUSINESS		106
ORDINARY MEETING 17th June, 1945.		
PAPER Pulmonary schistosomiasis. M. Erian		160
DISCUSSION The President (Sir Philip Marston-Baker); Sir Henry Tilly; Prof. Day; C C. Chatterman W Alves; Prof. Erian (in reply)		173
COMMUNICATIONS		
Blackburn G. H. Eickstein. Observations on the development of resistance to vivax malaria		117
Payne, Eugene H., Shary E.A., and Knaul, Jose A. Treatment of epidemic typhus with chloromycetin		123
Wildey, P. H. A. Malarial fever in Assam and Burma, 1944-46		171
Detrick, E. H. A fatal case of generalized amoebiasis due to protozoan closely resembling, if not identical with, <i>Leishmania</i> <i>hirsuta</i>		191
Barnes, M. E. Remarks of welcome to the Royal Society of Tropical Medicine and Hygiene. Washington, D.C., 14th May 1945		199
CORRESPONDENCE		
A note on the value of folic acid in the treatment of merozoite infections in Assam tea garden labourers. P. H. Shiris		203
Hatching speed of schistosome miracidia. Frank A. Goodfellow and D. M. Blair		206
COMPTONIA		206

CONTENTS

No 8 Issued 27th November, 1948

PAGE

ORDINARY MEETING	21st October, 1948	
PAPERS	The epidemiology of fungus diseases	James T Dunoon 207
	Treatment of fungus diseases of the skin	I Muende 216
DISCUSSION	G C Alnsworth, Sir George McRobert, H S Stannus, J T Duncan (in reply), I Muende (in reply)	223
COMMUNICATIONS		
Shortt, H E	The life cycle of <i>Plasmodium cynomolgi</i> in its insect and mammalian hosts	227
Azim, M Abdel, and Ayyad, Nagulb	A preliminary report on the value of palm-leaf traps in the survey and treatment of streams infested with snails	231
Harrison, J L, and Woodville, H C	An attempt to control house rats in Rangoon	247
Bernkopf, H, Stuczynski, L A, Gotlieb, T, and Halevy, Ch	Serological examination of human and cattle sera from Palestine for the presence of antibodies against a bovine strain of <i>Leptospira</i>	250
Ransford, O N	Therapeutic extradural block in tropical ulcer	267
Dawson, J, Findlay, G M, and Ward, R D	A note on vitamin B complex deficiency states among Africans in the Gold Coast	277
Gelfand, Michael	The diagnosis of schistosomiasis in Southern Rhodesia by the rectal biopsy technique	283
Clark, Malcolm	Lymphostatic verrucosis in the Fort Hall District of Kenya	287
Cockburn, T A	<i>Balantidium</i> infection associated with diarrhoea in primates	291
Gebert, S	Notes on certain aspects of the action of DDT residual sprays, and on the partial treatment of dwellings as a means of anti-anopheline protection	295
Fourman, L P R	Effect of climate on the blood pressure in acclimatized subjects	299
OBITUARY	C M Wenyon, C M G, C B E, M B, B S, B Sc., F R S, President of the Society, 1945-7	303
CORRESPONDENCE	Bancroftian filariasis	T Wilson 305
	Dr C M Wenyon and the Royal Society of Tropical Medicine and Hygiene	
	By Sir Philip Manson Bahr	309

CONTENTS

No 4 Issued 27th January, 1949

PAGE

LABORATORY MEETING,	18th November, 1948	
DEMONSTRATIONS		311
ORDINARY MEETING,	9th December, 1948	
PAPER	A survey of physiological studies of mental and physical work in hot and humid environments	Guy P Crowden 325
DISCUSSION	Dr J S Welner, Sir George McRobert, Dr O B Alakija, Sir Henry Tidy, Lt-Col J C Watts, Dr J S Welner and Professor Crowden	336
COMMUNICATIONS		
Covell, G, Nicol, W D, Shute, P G, and Maryon, M	Studies on a West African strain of <i>Plasmodium falciparum</i>	341
Harding, R D	A yaws campaign in Sierra Leone	347
Passmore, R	Severe anaemia in Indian Sepoys	367
Goyal, R K	Standardization of daboia and cobra antivenines	381
Lewis, D J	The extermination of <i>Anopheles gambiae</i> in the Wadi Halfa area	393
Schweitz, J	Notes on endemic and acute malaria in Central African natives	403
CORRESPONDENCE		
	Notes on <i>Spirochaeta persica</i> from Palestine and spirochaetes of relapsing fever from the Western desert (Tobruk area)	Rivka Ashbell 409
	Lymphostatic verrucosis	T H White 410
	Vitamin B deficiency states	D Fitzgerald Moore 412
	Intestinal infections of primates	R E Rewell 413
	Fourth International Congresses of tropical medicine and malaria	Thomas W Cameron 415
SPECIAL ANNOUNCEMENT	Miss M Wenyon	416
LIEUT-COLONEL CLAYTON LANE		416

CONTENTS.

No. 8. Issued 27th March, 1948

408

ORDINARY MEETING 70th January 1949

PAPER Malignant melanoma (Kewshorker). H. C. Trewell	417
DISCUSSION Professor Himsforth; Dr Waterlow; Sir George MaRobert; Dr Alan McKenzie; Dr C. C. Chesterman; Dr Dean Smith; Brigadier J. Bennett; Dr L. E. Kayler; Colonel R. P. M. H. Karty; Dr H. P. Mares; Dr Trewell (in reply)	425

COMMUNICATIONS

Mackerras M. J. and Ercole Q. M. Some observations on the action of quinine, atabrin, and plasmoquine on <i>Plasmodium vivax</i>	453
Mackerras, M. J. and Ercole Q. M. Observations on the action of quinine, atabrin and plasmoquine on the gametocytes of <i>Plasmodium falciparum</i>	456
Cotter G. Hesel, W. D., Skye P. G. and Maryon M. Studies on West African strains of <i>Plasmodium falciparum</i>	465
Krisa, M. Krisa, M. Momen, A. M. and Davis, A. A. Chronic pulmonary schistosomiasis: clinical and radiological study	477
Thomson, F. Adam. Dietary deficiencies in children in the island of Viti Levu, Fiji	487
Nagaty H. F. and Kamaty A. F. The treatment of polycythæmia: record of one case treated with <i>ascaryol</i> infection	493
Kirk R. Studies in Leishmaniasis in the Anglo-Egyptian Sudan	501
Kotschalain Rudolf P. Observations on the epidemiology of infections with <i>Clostridium stercorale</i>	503

CORRESPONDENCE

Mechanical purification of river water F. Gordon Cameron	507
A case of recurrent haemoglobinuria treated with sulphathiazole J. O. Sitroon	509
Intestinal infection of primates T. A. Cookburn	509

CONTENTS.

No. 9. Issued 21st May 1949

408

ORDINARY MEETING 17th February 1949

PAPER The epidemiology of yellow fever in Central Africa A. F. Mahaffy	511
DISCUSSION Dr G. M. Findlay; Mr F. F. Mattingly; Professor B. Macgregor; Dr P. C. C. Garrahan; Dr C. J. Mahaffy; Dr W. D. R. Mac; Dr M. T. Morgan; Dr Mahaffy (in reply)	523

COMMUNICATIONS

Baylis A. H. A new human cestode infection in Kenya (<i>Isospora capensis</i> acanthocysts, parasite of rats)	531
Woodman, Hugh M. Filaria in the Anglo-Egyptian Sudan	543
Gottard M. and Ross, W. F. The incidence of schistosomiasis in South Central Africa	559
Blask, Robert H. Observations on the treatment of falciparum malaria	565
Field John W. and Köster, J. F. B. A note on presumed erythrocytic development of <i>Plasmodium reuteri</i> in the liver of the Malayan squirrel	568
H. Kestrop A., Soong Tienj Kien and Li, Young. Rate of disappearance of <i>Leishmania</i> in kala-azar patients under urea schistosomium therapy	573
Priest, W. M. Acute meningo-encephalitis of uncertain origin in West African troops	581
Armstrong, T. G. Wilson, A. J. and Rhodes-Dew R. The treatment of amoebic dysentery in the Bantu African	597
W. edrall A. W. Aetiological and prognostic features in tropical sprue	607
Corkill, N. L. Malnutrition and mask poisoning in the Sudan	613
Jaffé Ludwig. Scleroma (Rhinoscleroma)	617
Hughes, Mark. A case of thromboangiitis obliterans in an African woman	621

CORRESPONDENCE

The efficacy of paludrine (proguanil) therapeutic agent.	623
N. Hamilton Fairley	624
Electrical charge of trypanosomes. H. E. Harshy	625
Lymphostatic verrucosities. L. J. A. Leersenthal	625
Lymphostatic verrucosities. Malcolm Clark	629

The previous number of these Transactions, Vol 41, No 6
was published on May 26th, 1948

TRANSACTIONS
OF THE
ROYAL SOCIETY OF TROPICAL MEDICINE
AND HYGIENE

VOL 42 No 1 JULY, 1948

LABORATORY MEETING
of the Society held at the
Royal Army Medical College, Millbank, London
on
Thursday, 18th March, 1948, at 7 30 p m

THE PRESIDENT,
SIR PHILIP MANSON-BAHR, C M G , D S O , M D , I R C P ,
in the Chair

DEMONSTRATIONS

ROYAL ARMY MEDICAL COLLEGE

Lieut -Colonel A Sachs, Lieut -Colonel A N T Meneces, Lieut -Colonel
M H P Sayers, and Lieut -Colonel J J O'Dwyer
Scrub typhus

Sections stained by haematoxylin and biebrich scarlet from a fatal case of typhus in India due to *Rickettsia tsutsugamushi* infection were shown to demonstrate the characteristic lesions of the disease Photomicrographs of sections from a fatal case of typhus in Iran, due to *R prowazeki* infection, for comparison, demonstrated the similarity of the histopathology of the two infections As far as can be determined from brains of fatal cases of *R tsutsugamushi* infection examined in India, it is not usual to find the typical nodules in the brain described by WOLBACH in *R prowazeki* infection

Sections stained by alkaline Giemsa were shown to demonstrate *R prowazeki* in an egg-yolk culture of the Addis Abbaba strain (obtained from

CONTENTS

No. 5. Issued 27th March, 1949.

VOST

ORDINARY MEETING 20th January 1942

PAPER: Malignant melanotritosis (k.washlockor). H. C. Trowell 417
 DISCUSSION: Professor H. Washlockor; Dr. Waterloo; Mr. George
 McRobert; Dr. Alan M. Kucala; Dr. C. C. Chederman; Dr.
 Dean Smith; Brigadier J. Bennett; Dr. L. E. Hapner; Colonel
 E. P. M. H. Early; Dr. K. P. Hare; Dr. Trowell (in reply) 423

COMMUNICATIONS

Mackerras, M. J. and Eriqle Q. N.	Some observations on the action of quinine, streptomycin, and plasmoquine on <i>Plasmodium vivax</i>	113
Mackerras, M. J. and Eriqle Q. N.	Observations on the action of quinine, streptomycin and plasmoquine on the gametocytes of <i>Plasmodium falciparum</i>	115
Cereil G. Noel W. D., Kirke, P. G. and Maryon, M.	Studies on West African strains of <i>Plasmodium falciparum</i>	118
Eriqle, M. Eriqle, H. Hume, A. H. and Deek, A. A.	Chronic pulmonary schistosomiasis: clinical and radiological study	122
Thomas, F. Adam	Dietary deficiencies in children in the island of Val Lery, Fiji	127
Nagaty H. F. and Zaky A. F.	The treatment of polycythemia vera: a record of one case treated with <i>amniocentesis</i> selection	132
Kirk, R.	Studies in Leishmaniasis in the Anglo-Egyptian Sudan	201
Kotrlitzki Rudolf P.	Observations on the epidemiology of infections with <i>Chlamydia trachomatis</i>	203

CORRESPONDENCE

Mechanical purification of river water. F. Gerdien, Dawson	507
A case of recurrent haemoglobinuria: treatment with sulphathiazole J. O. Stittgen	508
1 (sexual) infection of primates. T. A. Caplan	509

CONTENTS

H. C. Award 27th May 1949

4

ORDINARY MEETING 11th February 1984

DISCUSSION Dr O. H. Finlay; Mr P. F. Maitland; Professor B. Macgregor; Dr P. C. O. Graham; Dr C. J. Hackett; Dr W. H. R. Razi; Dr M. T. Morgan; Dr Mahaffy (in reply)

COMMUNICATIONS

Baylis, A. M.	A new human cestode infection in Kenya. <i>Trichocephalus axei</i> (Schrank), parasite of rats	531
Woodman, Hugh H.	Filaria in the Anglo-Egyptian Sudan	543
Oeldred, M. and Ross, W. F.	The incidence of schistosomiasis in South Central Africa	554
Mask, Robert H.	Observations on the treatment of falciparum malaria	565
Field, John W. and Kateson, J. F. B.	A note on presumed eosinophilic development of <i>Plasmodium vivax</i> in the liver of the Mexican squirrel	569
H. Entzang A., Soeng Tsung Hsia and Li, Tsung	Some of the appearances of <i>Leishmania</i> in skin-scar patients under urea sulphonic therapy	573
Priest, W. M.	Acute meningo-encephalitis of uncertain origin in West African troops	581
Armstrong, T. G. Wilmer, A. J. and Enkes-Dew N.	The treatment of amoebic dysentery in the East African	597
Westcott, A. W.	Articulation and prognostic features in tropical sprue	605
Corkill, E. L.	Misadventure and snake poisoning in the Sudan	612
Jaffé, Ludwig	Sclerosis (Rhinoscleroma)	617
Hughes, Mark.	A case of thrombotic thrombocytopenic syndrome in an African	621

RESPONSE

The efficacy of paludrine (proguanil) as	therapeutic agent	623
M Hamilton Fairley		624
Electrical charge of trypanosomes.	M. E. Herby	624
Lymphostatic verrucosa.	L. J. A. Leewenthal	625
Lymphostatic verrucosa.	M. J. Clark	625

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Mr J. H. Grundy

Entomology of scrub typhus

Demonstrated the life-cycle of the important vector, *Trombicula deliensis*, in the following stages Unfed larva, gorged larva attached to "feeding tube" of host's tissue, nymphochrysalis, nymph, imagochrysalis, adult

An egg was shown within the hind body of *Ascoschongastia indica*, 2/3 inch objectives were used on the microscopes, and a magnification of 130 for mite-representation on the original coloured chart of the life-cycle

A specimen of the bandicoot rat, *Bandicota malabarica*, an important animal host in the S W Pacific and S E Asia region, was shown together with a collection of rodents' ears with *Trombicula* larvae attached

The use of slide-preparations lent by Sqdn-Leader C D RADFORD is gratefully acknowledged

Lieut-Colonel F Buckland

Colony counter

The apparatus comprises a pen-holder made of plastic

The nib is attached to a central rod which is surrounded near its distal end by a silver band wired to the positive pole while a silver band fitted to the corresponding position inside the casing is connected to the negative pole

When pressure is exerted on the nib the central rod flexes, the two bands make contact and complete the circuit The pen is wired to an electro-magnetic post office recorder which, in turn, is connected to the D C supply, through a suitable resistance, to give a current of 50 volts

The apparatus is fitted into a box with a perspex window on to which the petri dish fits illumination is provided by a light bulb and the colonies viewed by indirect light against a black background

Each colony is counted by making a mark in ink with the pen on the base of the petri dish the pressure required to do this causes contact to be made in the pen-holder and the recorder to be activated

Messrs Evans

Evans-electric portable photo-electric colorimeter *

This new photo-electric colorimeter is a completely self-contained instrument, incorporating its own 2-volt power supply Attractively finished in a black and chromium cast aluminium case, this apparatus is ready for instant use under field or laboratory conditions

Due to progress made in development, it now becomes possible to produce a really reliable photo-electric colorimeter, capable of consistent results over an

* The "Eel" portable colorimeter is manufactured by Messrs Evans Electroselenium, Ltd, Harlow, Essex.

indefinite period of time. Besides being eminently suitable for haemoglobin determinations, this particular colorimeter may be used to compare almost any colour liquids with unusual accuracy free from human error and eye fatigue

Professor E. J. King

A simple portable photometer for haemoglobinometry and other colorimetric analyses.

The photometer consists of an accurately calibrated annular neutral grey wedge, a section of whose field is brought into juxtaposition with the haemoglobin red colour by means of an eye-piece. By means of a spectral filter green light only is allowed to traverse the red solution and grey wedge and to reach the eye. This green light is strongly absorbed by the red solution, which causes that part of the field which is due to light passing through the solution, and which is seen through the eye-piece to appear darker than the other half of the field. The green light appearing in this other half of the field is diminished by rotating the wedge and bringing an increasingly denser part of the grey into the field, until the intensity of the green light appears equal on both sides. The

photometric end point "or" match, may be approached from either side, i.e. from darker to lighter as well as from lighter to darker. By rotating the grey wedge back and forth over the end point an accurate match may be obtained. This is best accomplished by holding the photometer squarely in front of a brightly illuminated window or a strong electric light. It must not be held obliquely to the light source. If the light enters the instrument at an angle it will not illumine the two sides of the field equally. An artificial light attachment is supplied as an adjunct to the photometer.

The circular grey wedge is divided into a haemoglobin scale and the point of matching is recorded in terms of percentage of Haldane. Several readings are taken, the number depending on how well they agree, and the average is recorded. If so desired, the reading may be turned into grammes of haemoglobin per 100 ml. of blood, or into "optical density D" (or extinction E) by means of the factors furnished with each instrument. Since the colour of haemoglobin obeys Beer's law in mono-chromatic green light, the optical densities of the solutions will be such as to furnish readings which are in simple arithmetic relation to their concentrations. The greater the concentration (i.e. grammes of haemoglobin) the higher will be the reading. In fact, doubling the concentration will double the reading.

The instrument can be used for any other colorimetric estimation as well as haemoglobin, e.g. blood urea or blood sugar. For these estimates ordinary test and standard are read in the photometer exactly as for haemoglobin. The green filter serves for the blood urea Nessler colour and the red filter for the blood sugar blue colour.

The calculation consists simply of factoring the reading of the unknown test solution by that of the known standard solution, and multiplying by the concentration of the latter. Thus, using this instrument to measure the colour in each of these estimations, the calculation is similar to that when a Duboseq colorimeter is used save that the first fraction is inverted, the standard reading becoming the denominator and the test reading the numerator.

Adherence to Beer's law by solutions of Nessler yellow holds only over a small range of concentrations. If test and standard differ grossly, *i.e.*, by more than 100 per cent, it is necessary to prepare another standard more nearly equal to the test. Attainment of proportionality is assisted by placing the "blank" colour solution in position behind the grey wedge to compensate for the light colour due to the reagent itself. This compensation with a blank is also advisable in the case of blood sugar.

The grey wedge photometer is described in full by KING *et al* in *The Lancet*, 1948, where a full discussion is given of accurate methods of estimating haemoglobin and of the performance of various instruments. Messrs. Keeler Optical Products, Ltd., of 39, Wigmore Street, W.1, are the manufacturers.

Major H. M. Rice

Hydrophobia, becoming overt in the United Kingdom

Patient developed rabies 6 weeks after a bite on the hand, from a "mad" dog, in Greece. Full course of fourteen injections of antirabic vaccine started within 10 hours of the bite. First symptoms appeared on 1.12.46. Fever started on 6.12.46. Diagnosis unmistakable, 8.12.46 (date of admission to hospital). Patient died, 9.12.46.

Diagnosis confirmed by histological examination of brain. Rabbits inoculated intrathecally with brain-emulsion developed paralysis on the 17th day, and Negri bodies were demonstrated in their brain sections.

DEMONSTRATION

(1) Two slides of Negri bodies in a dog's brain (stained H. & E.) showing a very heavy infection.

(2) Two slides of material from a human case quoted, showing scanty Negri bodies in the hippocampus. (Stained with iodized eosin and methylene blue.)

(3) Two photomicrographs from the slide of human rabies, showing Negri bodies.

(4) A slide (human) showing masses of white cells in the capillaries of the mid-brain, with no evidence of thrombosis, many of the cells being histiocytic in appearance.

For comment.

(5) A slide of rabies (canine) stained with Field's stain, in which the Negri bodies showed pale blue. This slide and the first two demonstrate the ease with which some Negri bodies can be stained.

LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE.
DEPARTMENT OF ENTOMOLOGY

Dr J. R. Burvine,

A simple field method for detecting DDT residual deposits.

Origin. Based on the colorimetric method of SCHLICHTER and HALLER (1944 *J Amer chem. Soc.*, 66 2129) and modified for field use in the Sardinian mosquito eradication campaign by Dr M. E. ALEMANDRINI. The object is to detect residual DDT deposits on walls supposed to have been treated.

Method.

- (1) Collect scrapings from surface area of about 25 sq. cm.
- (2) Mix 5 ml. conc. H_2SO_4 with 7 ml. conc. HNO_3 pour 3 c.c. of this mixture on to the scrapings and boil gently for 2 minutes (sparit lamp).
- (3) Cool. Add slowly with shaking about 8 c.c. water (until no more nitrous fumes are evolved).
- (4) Pour into separator funnel and add 1 to 2 c.c. benzene shake and discard aqueous layer.
- (5) Pour the benzene into clean tube, add an equal quantity of 8 per cent. alcohol KOH and warm just to boiling point. Colour indicates DDT (ranging from pale greenish blue (0.1 mg.) to deep purple red (10 mg. or more)).

Dr Rajindar Pal

Apparatus for studying the behaviour of mosquitoes in darkness

A technique for marking mosquitoes with fluorescent compounds and watching them by ultra-violet light has been developed to study the movements of these insects at night without affecting their normal behaviour (PAL, 1947 *Nature* 160 30th August, 1947). The mosquitoes are dusted with anthracene, photophour or standard "maradulux" powders 125-watt "mecca" lamp, with a concentrating reflector is used for ultra-violet light. The dusting procedure is very simple. The mosquitoes are placed in a glass chimney and given a few puffs of the fluorescent dust by means of a hand duster. The method seems very promising and opens out possibilities for the study of the ecology and bionomics of mosquitoes hitherto not possible.

Dr W. S. Richards.

The life history of the British harvest mite *Trombidium autumnale* with a description of sampling methods for larvae

They occur most abundantly on chalk down, though by no means confined to these localities. The pre-engorgement larvae may be collected, using bat animals (the best are guinea pigs) confined in light folding cages, or by means of simple light traps which consist of a cover with a central hole capped by a

British Thomson-Houston Co., Ltd.

glass collecting tube Larvae trapped beneath the cover crawl towards the light shining through the central hole and are collected in the tube

The larvae feed on most warm-blooded animals, *e.g.*, hedgehog, stoat, squirrel, feeding not on blood but on an area of degenerating epidermal tissue (histiosiphon) induced by the bite

The six-legged orange larva, after gorging, goes down into the soil and develops into the eight-legged nymph, distinguished by its dense covering of white-plumed hairs and constricted waist The adult is similar but larger

DEPARTMENT OF PARASITOLOGY

Professor H E Shortt and Dr P C C Garnham.

† Successive stages in the pre-erythrocytic cycle of *Plasmodium cynomolgi* from the fifth to the eighth days of the incubation period

Fifth day A small schizont 10.5μ in diameter, situated in a liver cell

Sixth day A schizont 23μ in diameter containing very numerous particles of chromatin, situated in a liver cell

Seventh day (a) A large schizont, 28μ in diameter, containing very numerous particles of chromatin (b) A very large schizont measuring 30μ in diameter containing a very large number of particles of chromatin and showing a large, sharply outlined vacuole (c) A large schizont which contained over 300 particles of chromatin, seen in a liver smear

Eighth day A large schizont measuring 30μ in diameter with very numerous particles of chromatin The nucleus of the containing liver cell indenting the parasite

The pre-erythrocytic stage of *P. vivax* on the seventh day of the incubation period

(a) Low-power view of schizont in the liver showing a central vacuole

(b) A large schizont measuring 35μ in diameter containing numerous particles of chromatin The parasite is somewhat shrunken away from the surrounding liver cells

LIVERPOOL SCHOOL OF TROPICAL MEDICINE

DEPARTMENT OF ENTOMOLOGY AND PARASITOLOGY

Professor R M Gordon and Mr W Crewe

Man's reaction to the bites of *Aedes aegypti*

Graphs and colour photographs were shown which illustrated man's reaction to mosquito bites and the alterations observed in these reactions following further exposure of the individual to irregular biting by *Aedes aegypti*

† A fuller account of this demonstration is given in these TRANSACTIONS, 1948, 41, 785

During the act of feeding the mosquito introduces a substance into the tissues of the host which in the majority of individuals causes a reaction which does not develop until some considerable time after the bite. This type of reaction is referred to as "the delayed reaction" and, for want of a better term, we call the substance producing it a "toxin." If persons showing a delayed reaction to *Aedes* are irregularly exposed to further bites from the same species, a proportion become sensitized, i.e. they show an "immediate reaction" and the delayed reaction ceases to occur. If however they are regularly bitten at short intervals they do not become sensitized and the delayed reaction becomes progressively less intense and of shorter duration.

It is suggested that the "immunity" to mosquito bites generally exhibited by natives of all ages in the tropics is not a racial characteristic but is due to the fact that from an early age they are regularly exposed to the bites of these insects.

If persons showing an immediate reaction to *Aedes* are irregularly exposed to further bites from the same species the sensitivity persists for an indefinite period. If however they are regularly bitten at short intervals the duration and intensity of the immediate reaction become progressively though irregularly less, the start of the reaction remaining immediate. The sensitization described is most marked locally: thus a person who has been recently desensitized to *Aedes* by repeated feeding on one area of the body will still react on other parts of the body although to a lesser extent than before the desensitization. Desensitization as well as sensitization, appears to be highly specific: thus an individual sensitized to *Aedes Culex*, and *Anopheles*, and subsequently desensitized to *Aedes*, still gave an immediate reaction to the bites of *Culex* and *Anopheles*.

In the case of sensitized and non-sensitized persons taking "benadryl" and exposed to mosquito bites, no appreciable change was observed in the immediate or delayed reaction, but a noticeable diminution of itching was recorded in each instance. In distinction from the failure of "benadryl" to control mosquito bites, this drug markedly reduced the reaction of an individual highly sensitized to tsetse bites.

The literature concerning man's reaction to insect bites is extensive, and most, although not all, of the points recorded in this demonstration have been the subject of studies by previous workers.

Dr D S Bartram and Miss E W Roberts

A technique for investigating the effectiveness of insecticides against *Dermatobia (Borocela) boris* L. the biting louse of cattle

During investigations in 1946-47 on the effectiveness of insecticides against the biting louse of cattle, *Dermatobia (Borocela) boris* L. it became apparent that visual assessments of the intensity of infestations were inadequate as criteria for comparative work. A sampling technique was devised as described below

and has been found to be effective in enabling comparisons to be made throughout the winter of 1947-48 on the effectiveness of different applications of DDT and "Gammexane"

A piece of surgical lint (18 × 12 inches), placed on the sacral region of an animal is overlaid by an electric pad heated to approximately 30° C by connection to the normal lighting circuit. The pad is left on the animal for 2 minutes, the temperature being checked by inserting a thermometer between the lint and the animal's hide. The pad is then withdrawn, the lint removed, and the number of lice adherent to it is taken as a representative sample of the louse population on the animal. Such a sample is referred to as a "lift". "Lifts" have been taken from the sacral region, withers, neck, and belly. The number of lice is counted at once, or the lint is transferred to a labelled envelope and the "lift" counted at leisure.

"Lifts" from infested animals may contain from a few up to 2,000 lice according to the degree of infestation. After insecticidal treatment a re-establishment of the louse population from surviving eggs, and the gradual growth of the nymphs to the adult stage have been traced by "lifts" at intervals over a period of 5 weeks.

DEPARTMENT OF TROPICAL MEDICINE

Dr Robert H Black

Paludrine forms of Plasmodium falciparum

A series of slides was shown demonstrating, firstly, the appearance of *P. falciparum* developing in a control culture containing no drug in the serum used for the medium and, secondly, the changes seen in the parasite when the serum of the culture medium is obtained from a healthy volunteer 4 hours after the oral administration of 100 mg of paludrine.

The parasites in the control culture underwent schizogony and formed rings of the second generation. In the culture containing paludrine development of the parasites ceased in the early schizont stage before the division of chromatin, they then developed vacuoles and degenerated—"paludrine forms".

Dr J D Fulton, Mr L P Joyner and Dr Janet Niven

The cotton rat as experimental host for kala-azar

It has recently been shown (*Jl Gen Microbiology*, in press) that the cotton rat is a suitable host for the experimental study of kala-azar. The infections are progressive in character and show no tendency to spontaneous regression for a period of at least 8 months. The new host possesses certain advantages over the hamster, being larger when fully grown and breeds well all the year round. Whereas in the hamster death is the invariable sequel to infection, the cotton rat appears to remain well in spite of heavy infections over long periods. Its value in chemotherapeutic tests is at present being assessed.

- (1) Section of cotton rat liver showing *Leishmania*.
- (2) Section of cotton rat spleen similarly infected.
- (3) Spleen smear of heavily infected cotton rat.
- (4) Liver smear of infected cotton rat.
- (5) Section of hamster liver showing amyloidosis.
- (6) Section of hamster kidney showing amyloid infiltration.
- (7) Cotton rat with infection of some months duration showing the condition of internal organs, enlarged liver and spleen normal kidney and absence of peritoneal fluid.
- (8) Hamster with infection of some months duration showing condition of internal organs, generalized amyloidosis, with marked involvement of the kidney oedema, and ather.

Dr F Hawking Dr W L M. Perry and Miss J P Thurston

Tissue forms of *Plasmodium cynomolgi*.

Sections and photographs were demonstrated showing tissue forms of *Plasmodium cynomolgi* in the livers of three monkeys which had been injected intravenously with sporozoites 5 days, 7 days 15 hours, and 7 days 23 hours earlier respectively. The parasites develop in hepatic parenchymatous cells which become greatly distended in the process. The largest form found measured $68 \times 81 \mu$.

Dr W L M Perry Mr P F J Sewell and Dr F Hawking

Preservation of pathogenic organisms in a frozen condition for several months

A demonstration was given of the equipment for the preservation of organisms at -78°C . This consists essentially of a large thermos jar filled with solid carbon dioxide and alcohol. The fluid containing the organisms is placed in small ampoules which are kept immersed in this freezing mixture. Microfilariae and adults of the filarial worm *Latemosoides curru* were demonstrated which had been frozen for 2 months on thawing many of them showed motility again. Trypanosomes and *Trepomna duttoni* can be preserved in the same way.

MINISTRY OF AGRICULTURE AND FISHERIES, WEYBRIDGE

VETERINARY LABORATORY

Mr J G Brotherton

The effect of relative dryness on the oöcysts of *Eimeria tenella* and *E. bacis*.

Eimeria tenella Unsporulated oöcysts collected from the caeca of chickens were isolated and maintained at 29°C . in atmospheres of known relative humidities for periods of 45 and 65 hours. Oöcysts sporulated in an atmosphere as low as 60 per cent. relative humidity were found to be infective to chickens.

LABORATORY MEETING

in spite of the dryness having caused their outer envelopes to become broken and the sporocysts released, as was clearly shown in the photomicrographs which were exhibited. Specimens were also shown of chicken's caeca infected by oöcysts sporulated at 80, 90 and 97 per cent. relative humidities, although on microscopical examination they had appeared to be dead.

Eimeria bovis Photomicrographs of oöcysts maintained in atmospheres of 30 and 97 per cent. relative humidities showed that those at the lower humidity were contracted, the wall was ruptured, and there was no sporulation. At 97 per cent. relative humidity the oöcysts were distorted and there was collapse of the wall, accompanied by its rupture, but released spores could be seen in many instances.

Mr C Horton-Smith

The effect of sulphamezathine on the second generation schizonts of *E. tenella*

Caecal coccidiosis caused by the coccidium *E. tenella* is the second important disease of young chickens. *E. tenella* attacks the distal portion of the chicken's caeca. The rapid growth of the second generation schizonts is responsible for the collapse of the epithelial layer, tunica propria and submucosa. Severe haemorrhage results and the flow of blood into the lumen of the caeca carries many merozoites with it, and these proceed to penetrate other chickens. On the fifth day of infection, when most of the chicks die, much of the caecal wall has sloughed.

The stage in the life-history of *E. tenella* that is susceptible to sulphamezathine is the second generation schizont, but the action of the drug is so immediate as to interfere with the development of immunity which is initiated by this particular stage of the life-history.

Microscopical preparations and photomicrographs showed second generation schizonts in the caecal walls of untreated and treated chickens. Chickens were shown of the caeca of infected chickens which did not receive treatment with 0.2 per cent. sulphamezathine until 2 and 3 days after administration of oöcysts. The effect on the schizont of this drug, which produces blood concentrations of over 10 mg per 100 ml, is to inhibit nuclear division and segmentation into merozoites. If the treatment is initiated 2 days after the administration of oöcysts, the length of the schizonts is 28μ , but if treatment is delayed for 3 days the length is 30μ . The schizonts in untreated control chickens have a length of 30μ . Another series of slides showed sections of caeca of chickens treated with different dilutions of sulphamezathine. Chickens were shown of 0.025 per cent. to 0.1 per cent. in which the lengths of the schizonts were between 50μ in the 0.025 per cent. treated chickens to 25μ in the 0.1 per cent. treated chickens.

EYEBRIDGE

2nd E. bovis
caecal chickens

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Dr W L M Perry Mr P F J Sewell and Dr F Hawking

Preservation of pathogenic organisms in a frozen condition for several months.

A demonstration was given of the equipment for the preservation of organisms at -76°C . This consists essentially of a large thermos jar filled with solid carbon dioxide and alcohol. The fluid containing the organisms is placed in small ampoules which are kept immersed in this freezing mixture. Microfilariae and adults of the filarial worm *Latimeroides caroli* were demonstrated which had been frozen for 2 months on thawing many of them showed motility again. Trypanosomes and *Trepocerca duttoni* can be preserved in the same way.

MINISTRY OF AGRICULTURE AND FISHERIES, WEYBRIDGE VETERINARY LABORATORY

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LABORATORY MEETING

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treated chickens. There was a decrease in nuclear division and segmentation into merozoites when stronger solutions of sulphamezathine were used. The beneficial effects of sulphamezathine are bound up with this reduction in size of the schizont and the suppression of merozoite formation.

Mr A E Pierce

The demonstration of specific agglutinins to *Trichomonas foetus* in vaginal mucus.

The presence of specific agglutinins to *Trichomonas foetus* has been demonstrated in the vaginal discharges of infected cattle. The method consists of the mixing of vaginal mucus with melted agar which is allowed to solidify in small petri dishes. A suspension of live trichomonads is then pipetted on to the surface and the preparation incubated at 37° C. A qualitative assay is made by applying the scale used by ROBERTSON (1941) for judging the degree of agglutination. Agglutinins make an earlier appearance in the vaginal mucus than they do in the blood and promising results have already been obtained in the application of this test to the diagnosis of trichomoniasis among herds of cattle.

Microscopical preparations and photomicrographs were exhibited and showed the various degrees of agglutination obtained in the agar preparation of vaginal mucus.

Dr E L Taylor

A culture method for *Lymnaea truncatula* the intermediate host of the liver fluke *Fasciola hepatica*.

Water culture has proved unsatisfactory for this snail which is better regarded as a mud snail. The exhibit consisted of two rich cultures of the snail, the culture procedure being as follows —

(1) Clay is collected from natural habitat of the snail and is sterilized by heat in an autoclave.

(2) Water also collected from a natural habitat of the snail, is mixed with the clay until the whole is of the consistency of wet mud.

(3) The mud is then moulded into slope in shallow container of glass or earthenware and depressions made in its surface reproducing, in miniature, the footprints of snails.

(4) Water from the snail habitat, containing algae is then poured over the surface of the mud in sufficient amount to form small pool at the bottom of the slope.

(5) The top of the culture dish is then covered with glass and the medium left in glass house or near large window for 3 or 4 weeks for the algal inoculum to mature before the snails are introduced.

At weekly intervals small amount of mixture of equal parts of powdered natural chalk and fine oatmeal is sprinkled on to the surface of the culture as supplement to the natural food.

The culture should be kept in the light, and at temperature of 70° F to 80° F if rapid growth is desired.

LABORATORY MEETING

Professor K B Williamson

- (i) Confirmation of cuticle no hospitality to human malaria parasites
 - (ii) Flagellate origin and flagelliform character of young trophozoites
- (1) Photomicrographs of sporozoite-containing cysts, and of Ross's black spores in association with cysts and sporozoites of *Plasmodium falciparum* and *P. vivax*, developed in laboratory-bred *Culex bitaeniorhynchus* which had been pre-indicated as a possible host by its larval habitat being in pure water photo-synthetically oxygenated by *Spirogyra* sp (1927, *Malay Med J*, 2, 53), which supplies the larvae with fresh green food. Out of 44 of the culex fed upon donors selected because their blood was exceptionally rich in gametocytes (averaging seven per hundred leucocytes), guts of 45 per cent and salivary glands of 34 per cent were infected. These consolidated figures include relatively very few infections with *P. malariae*, in which one or two cysts and only two chitnized sporozoites were observed.

(2) Reproduction of figures by Ross (letter to Sir PATRICK MANSON, 28th June, 1898) and by MISSIROLI (1938, *Rev Paras* 2, 39), showing nuclear division and sporogeny of sporozoites, and of drawings and photomicrographs respectively by GIOVANNOLA (1934, *Rev Malar*, 13, 327), and KNOWLES and BASU (1934-35, *Indian J of Med Res*, 22, 443) of exflagellation of sporozoites' chromidia *in vitro*, compared with drawings demonstrating like behaviour *in vivo* in human blood and lymph of sporozoites experimentally matured in *C. bitaeniorhynchus*, and showing that the resulting chromatin-headed thread flagellates (constituting the final link in Grassi's prognosticated sporogenous developmental chain) give direct rise to juvenile trophozoites by assuming snake-like, straight (band), or curved (commas, rings, etc.) sedentary poses, young trophozoites being flagelliform rather than amoeboid or vacuolate structures, while slow maturation, corresponding to that of resistant resting spores, and deferred exflagellation comparable to germination of latent malaria, from which they derive provides a hypothetical explanation of the particles both incubatory and relapsing, irrespective of the location of the particles whether in blood or in internal organs. Drawings were shown illustrating the occurrence free in blood of these particles or derived thread flagellates in both early and late stages of experimental infection, compared with corresponding trophozoital forms depicted in THOMSON and ROBERTSON'S *Protozoology*.

Dr. G. M Findlay

A microfilm copy was exhibited of the very rare pamphlet, "An Essay on the Bilious or Yellow Fever of Jamaica," by JOHN WILLIAMS, published in 1750 at Kingston, Jamaica.

Only one copy of this work is known to exist in Great Britain, in the Library of the University of Edinburgh. There is another copy in the Library of the Surgeon-General, Washington, D C, from which the microfilm is taken.

A large dosage, approximately 0.25 gramme thrice daily by mouth for 18 days, was used. At the fifth day of treatment the amoebae disappeared from the pus, and by the twelfth day the discharge had ceased and the wound was quite healed. In step with this local improvement, the general condition of the patient improved so that fortnight after the course was completed, he was sufficiently well to be discharged from hospital. He was then prescribed a maintenance dose of 0.25 gramme chloroquine bi-weekly for 3 months.

So far there has been no relapse, and when last examined 2 months after discharge from hospital, he was looking and feeling well. He had gained 17 lb in weight, the wound was soundly healed, the respiratory movements were good, there was neither tenderness nor obvious enlargement of the liver, no amoebae were found in the stools and the blood count was normal.

ORDINARY MEETING
of the Society held at
Manson House, 26, Portland Place, London, W 1
on
Thursday, 15th April, 1948, at 7 30 p m
THE PRESIDENT,
Sir PHILIP MANSON-BAHR, C M G , D S O , M D , F R C P ,
in the Chair

PAPER
**DISEASES OF TROPICAL ORIGIN IN CAPTIVE WILD
ANIMALS**
BY

R E REWELL, M D , M R C P *
Pathologist to the Zoological Society of London

Since a large proportion of wild animals in captivity have been brought from tropical regions, it is to be expected that some will bring with them diseases which occur characteristically in such places. However, the proportion carrying these is small, for a number of reasons. The animals are usually caught by native trappers and sold to professional collectors, either private individuals, who sell their collections to zoological gardens, or officials of these gardens sent out for this purpose. Since regulations have been introduced in many localities for the protection of wild life, official approval of their activities must be obtained in many cases, e g , in all British possessions and the Belgian Congo. These collectors are experienced men who do not purchase animals which are obviously diseased, while many which harbour unsuspected infections die from

* I have to thank the Zoological Society of London for permission to publish the records quoted. All the blood parasites mentioned were identified by Dr C M WENYON, and the helminths either by Dr ANNIE PORTER, the Zoological Society's Honorary Parasitologist, or at the Department of Helminthology of the London School of Hygiene and Tropical Medicine, formerly under Professor R T LEIPER, now under Professor J C C BUCKLEY. I have to thank them for permission to quote their findings. The illustrations are by Messrs ILFORD, Ltd.

I have drawn heavily on the facts recorded over the past 25 years by my predecessors Sir HAROLD SCOTT and Col A E HAMERTON. These have been reported in their yearly Reports to the Zoological Society and published in its *Proceedings*. Individual mention of these has not been made in the list of references.

the inevitable rigours of the journey to their final home. On arrival in temperate climates a large proportion of tropical animals succumb within a few months, but some survive for periods of relatively great length. These are animals which either were healthy on arrival, or which have succeeded in overcoming any infection they may have brought, or in reducing it to subclinical levels.

Many tropical infections and infestations require for their spread a special intermediate host or vector which is not found in temperate climates. Obviously such conditions will not be transmitted in captivity. That the parasite might use new vectors is possible, but this does not seem to occur in practice although proof of its occurrence would be difficult and the fact might be easily overlooked. Moreover the concrete floor of the ordinary zoo den is far removed from the conditions under which comparable spread of parasites can occur in temperate regions, e.g. the liver flukes of sheep, although it is conceivable that private herds of wild animals might infest with an exotic fluke the snails of some English water meadow.

Even when no such intermediate aid is required, spread of parasites is prevented to a large degree by the scrupulous cleanliness adopted in zoological gardens, especially here and in the U.S.A. Moreover another individual of the same species as the host, or one closely allied, must be in close proximity at the right time. This is likely not to happen, especially as many animals must be segregated in quarantine on arrival the time when parasites are probably most numerous and active.

When all allowances have been made it still seems curious that some conditions, common enough in the wild, should not be found in captivity. A good example is schistosomiasis in the gnu (wildebeest). This could not be expected to spread in captivity but it does not appear to have been recorded in temperate climates, even in animals recently imported. Once again, it is likely that infested animals die before being shipped from Africa.

In the London Zoological Gardens observation of many conditions is rendered difficult by an absolute ban on the taking of blood films from live animals except when symptoms are present strongly suggestive of a blood infestation. Faeces are collected from all newly arrived mammals and examined for the presence of worms and ova. A very large proportion of animals are found to harbour worms of some sort, only those bred in the Gardens are expected to be exempt.

DEFICIENCY CONDITIONS.

So little is known about the effects of nutritional deficiencies in most animals that these conditions are seldom diagnosed. It is surprising how little is known about the normal diet of some animals. For instance, it is only recently that the rare indrisine, *Arctocebus* has been discovered to be largely carnivorous and not vegetarian (SANDERSON 1941). Some animals with unusual diets die after a short time in captivity with no obvious signs of any deficiency e.g. the leaf eating monkeys of such widely separated genera as *Alouatta* and *Colobus*. On

E. I. IRWILL

the other hand, some animals appear to thrive on diets very different from their natural ones, e.g. the lorises, *Loris*, and some apes and Hyakodex. Naturally, the more frequently-encountered condition of debility is one which in man is not tropical at all, i.e.rickets, although nowadays this is not seen often owing to the liberal use of fish liver oils and irradiated food.

Recently, however, I have seen two conditions in Primates which bore a strong resemblance to deficiencies seen in man in the tropics. One was in a pig-tailed monkey, *Macaca nemestrina*, which developed intense infection of the conjunctiva of the lids and eyelids resembling that seen in human trachoma, as well as redness and evident soreness of the skin round the anus. The animal was given large quantities of coconut which is rich in riboflavin, and the condition improved. It relapsed when the coconut was stopped but improved once more when the diet was supplemented again.

The other case was in a Sumatran gibbon *Symphalangus* which was imported from the Far East by a private collector. On arrival in England the animal was very thin, with a dry skin covered with fine wrinkles and from which most of the hair had fallen leaving some areas bare and others only with broken stumps. It was very tame as were several other gibbons of different species without these appearances brought in at the same time. Comparison with these showed the Sumatran to have absent knee tendons and triceps jets and possibly some incapacity to pick up on the extremities. A diagnosis of dry beriberi was made. Unfortunately the animal died before treatment could get under way and necropsy was refused by the owner, who got a good price for the unopened body as an anatomical specimen.

Microscopic intestinal lesions are encountered in many birds and some mammals, especially musksheds and bears. Macroscopic intestinal lesions are apparently due to dietetic deficiencies, as seen in the Sumatran interster, *Tamandua tetradactyla*. This animal lives on a very specialized diet of ants without any dilution of earth or other debris picked up from holes which it tears in tree trunks. This diet must contain huge quantities of formic acid, among other things. Symptoms of sprue do not appear.

BACTERIAL INFECTIONS

Many of the types of pathogens of man in the tropics occur, though not always producing similar lesions. Thus, organisms of the genus *Pasteurella* are common in rodents, causing pneumonia and septicaemia. Recently such an organism destroyed the entire colony of prairie marmots, *Cynomys ludovicianus*, at the London Zoo. Some strains are remarkably specific for a particular species of host, as a number of African squirrels in the same cage in an outbreak killed all the white-spotted ground squirrels, *Funisciurus leucostigma*, and left the others.

True dysentery caused by human pathogens and with identical lesions occurs in the great apes. In monkeys, septicaemia with mild enteritis is caused. In Table I are shown the records of such cases in the English literature. Other

outbreaks have been recorded in France and Germany. From most cases of enteritis in animals, no recognized pathogen is isolated, however.

Of the salmonellae *S. ankyra* type mutton has been reported in baboons, causing an outbreak of enteritis with carriers (SCOTT 1927) and this organism

TABLE 1

RECORDS OF THE OCCURRENCE OF HUMAN DYSENTERY AND NERVE IN OWLS FROM THE IV IN ENGLAND.

SCOTT (1927).	<i>Campylobacter</i>	<i>B. flexneri</i> .
LOVELL (1929).	<i>Campylobacter</i>	
FATHERBROTHER and HERT (1922).	<i>Shigella sonnei</i> .	<i>Shigella flexneri</i> W. H. A.
		1 three,
		schubertii
		1 Strong bacillus.
REWELL and BRIDGES (1911).		<i>Shigella flexneri</i> 103 Boyd, three

under its later name *S. typhi muensteri*, has been similarly reported in penguins (COCKBURN, 1947). In sheep this organism may cause a choleraic condition, even in temperate climates, and is well known as a cause of septicæmia in many rodents. Salmonellae of various species have been found in different reptiles, especially by EDWARDS in the U.S.A. and recently Dr JOAN TAYLOR, Miss DOUGLAS and I have found a new species in an African python, *Python sebae* with an ulcerative condition of the intestine (unpublished).

SPIROCHAETES AND RICKETTSIAS.

These are not encountered in captivity. I have performed the Weil Felix reaction on the blood of several mammals dying soon after arrival from Africa, but with negative results.

VIRUSES.

Once more only a limited selection of conditions known to occur in the wild are seen in captivity. Rabies and the neurotropic viruses of monkeys, for instance are not seen. Psittacosis is one of the rare examples of a natural infection of wild animals in which classic work was done in captivity (GORDON 1930 and TROUP *et al.*, 1939). Nowadays parrots can be imported only by special licence and rigid quarantine is carried out. Since the outbreak in 1939 when one keeper died, the Zoological Society of London has been allowed to import many parrots from all parts of the world where they occur but no clinical case of psittacosis has been found among them, nor have inclusion bodies been found in the spleens of any which have died.

FUNGUS

Actinomycosis of bovines is well known. A streptothrix infection known as nocardiosis kills almost half of the captive wallabies in all parts of the world.

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The commonest site of origin is the buccal cavity or nose. The mucosa is eroded and the bones of the jaw or palate involved, so that the latter may be perforated. The condition may spread to the lungs or glandular area of the stomach and thence to the pancreas, liver and peritoneum (Scott, 1925)

I have seen in outbreak of mycotic pneumonia in wallabies due to *Aspergillus fumigatus*, and one case of a lung infection with this fungus in an American bison, *Bison bison* (RWHIT and AINSWORTH, 1947)

Birds from all parts of the world frequently have fungal infections of the lungs and air-sacs

PROTOZOAN PARASITES

In dead animals many parasites are found which were obviously in no way responsible for the death. Table II shows the parasites from the blood of dead animals identified by Dr C M WERNON during a year when many new ones were admitted. The small proportion infected needs no emphasis

TABLE II
TABLE SHOWING BLOOD PARASITES IDENTIFIED BY DR C M WERNON IN
ANIMALS DYING DURING THE YEAR 1917

	Mammals	Birds	Reptiles
Haemogregarines	0	0	27 (3)
Haemoproteus	0	0 (1)	0
Plasmodium	0	5 (1)	0
Leucocytozoon	0	5	0
Babesia	0	1 (1)	1 (1)
Microfilariae	0	0 (1)	0
Double Infections	2 (1)	7 (1)	
Totals	3	27	28
Number of necropsies	730	432	300

The figures in brackets show the numbers in which death was attributed by the writer to the infestation

Haemoproteus and *Leucocytozoon* in birds and haemogregarines in reptiles are common and of no significance. African ungulates may harbour *Babesia* for years, and this again seems to cause no trouble in most cases. However, the one case shown in Table II was of a young reticulated giraffe, *Giraffa reticulata*, recently imported from Kenya. It died after a pyrexial illness lasting about a week, while at necropsy the only obvious abnormalities were enlargement of the lymph nodes and of some of the lymphoid patches in the intestine. No other parasite or organism was found to account for this, so it is possible that the *Babesia* had gained an unusual advantage owing to the unnatural conditions under which the host was living, and might be held responsible for its death.

It is well known that where trypanosomiasis is enzootic in cattle severe environmental conditions will lead to an increase in deaths.

In Tables III and IV are shown the mammals in which plasmodia and microfilariae were identified over a period of 15 years. Only mammals are taken, as being of most interest to medical men and veterinarians. It will be seen that very few were encountered, although some 300 mammals were examined each year. Actually plasmodia are found most often in birds, including such unexpected species as penguins. These birds occur in far warmer regions than

TABLE III.
RECORDS OF PLASMODIA IN MAMMALS FOR THE
YEARS 1925-1939.

<i>P. circumscripta</i> .	<i>Peromyscus leucopus</i> .
<i>P. pitheci</i> .	<i>Peromyscus</i> , 10 cases.
<i>P. harti</i> .	<i>Citellus arizonae</i> .
<i>P. pectori</i> .	<i>Peromyscus</i> .
<i>P. cincti</i> .	<i>Citellus</i> 11 per cent three cases.
Unidentified.	<i>Hylomys</i> .
	<i>Peromyscus</i> .
	<i>Schistosoma</i> .

TABLE IV
LIST OF MAMMALS IN WHICH MICROFILARIAE WERE FOUND
DURING THE YEARS 1925-1939

<i>Antropomys</i> .	<i>Felis</i> .
<i>Citellus</i> .	<i>Felis</i> .
<i>Citellus</i> .	<i>Citellus</i> .
<i>Salmis</i> .	<i>Ovis</i> .
<i>Leontideus</i> .	<i>Puma</i> .
<i>L. rosalia</i> .	<i>Artibeus</i> .
<i>Haplo</i> .	<i>Nandua</i> .
<i>Larus</i> .	<i>Canis</i> .
<i>Nyctalus</i> .	<i>Babirusa</i> .
<i>Felis</i> .	<i>Dromedary</i> .
	<i>Procyon</i> .

is popularly supposed, and all those in which the parasites have been found appear to have come from outside the Antarctic Circle. In London no case of spread of plasmodia by native mosquitoes has been suspected.

As well as the parasites mentioned above, *Leishmania* is found sometimes in reptiles. Trypanosomiasis is rarely encountered, especially in mammals. However one case of great interest occurred in 1945 when *T. congolensis* was found in the blood of a West African water-buck, *Tragelaphus* imported from Nigeria in 1934. Thus the parasites had persisted for 11 years in an animal removed from a tsetse-fly area, even in the absence of a mechanical vector.

Leishmania is not found.

A huge variety of ciliates and other protozoa are found in the intestines

R E REWEIL

of mammals, the lungs of reptiles and other situations They may be associated with local lesions, but their pathogenicity is often very doubtful Balantidia are found sometimes in the faeces of Primates with diarrhoea I have seen them in the pig-tailed monkey, *Macaca nemestrina*, and in the orang-utan, *Pongo pygmaeus*

CESTODE INFESTATIONS

These are not quite so frequent as nematodes, but most groups of animals are involved All sizes are encountered, from the large taeniæ of the Canidae to the small members of the genus *Hymenolepis* which are found in many groups, including Primates In the Hyracoidea, infestation with cestodes of a relatively enormous size is almost the rule

Cestode cysts are very common in old animals, when they are usually too degenerated for identification to be possible I have seen many such cysts in material even from whales Among others, coenurus encysted in the brain of ungulates may produce fits and other signs of a space-occupying lesion The occurrence is rare, but I have recently seen it in a blackbuck, *Antelope cervicapra*

Flukes are too exacting in their demands for special intermediate hosts to be encountered very often However, pulmonary paragonimiasis in a tiger, *Felis tigris*, has been seen at the London Zoo, while liver flukes are sometimes encountered in Equidae and Bovidae

FILARIAL INFESTATIONS

These are most common in birds, although mammals and reptiles are certainly not exempt Some birds harbour filariae almost invariably Thus, worms of the genus *Diplotrinaena* occur in the air-sacs of birds, but in the occipital blue pie, *Urocissa melanocephala occipitalis*, their presence is to be expected As so often with wild animals, the infested birds die most suddenly and unexpectedly

In mammals, the Canidae seem most prone to filarial infestation No elephantiasis is ever seen

Recently I have seen three Meller's chameleons, *Chamaeleon melleri*, with heavy blood infestations with microfilariae, and a number of adult worms under the skin Identification of these adults is not yet complete

NEMATODE INFESTATIONS

These may involve almost all groups of animals and are often very severe Thus the lungs of snakes may present an appearance reminiscent of Medusa's head, the intestines or bile ducts of the pig tribe may be blocked with masses of ascarids, or the crops of birds by *Sporozoa* Worms may live in air-passages, e.g., *Syngamus trachealis* in many birds, in ligaments, e.g., *Onchocerca* in the Bovidae, under the skin of Carnivora or in the frontal air-sinuses of some mustelids Other curious sites where worms are found only in certain groups of hosts include the aneurysms of the mesenteric vessels or aorta of Equidae

caused by *Strongylus vulgaris*, and the frequent presence of *Heterakis* sp. under the mucosa of the caeca of pheasants of different species, sometimes without obvious lesions, and sometimes with chronic inflammation and ulceration (Figs. 2 and 3).

Far too many species are encountered for a summary to be possible. The number of species of parasite recorded from any host depends largely on the thoroughness with which this has been studied. Thus, even in domestic animals NEVEU-LEMAIRE (1949) lists thirty-one nematodes as having been found in the intestine of the horse, but only one from the yak, *Poephagus graxiensis*.

Most of the types of disease seen in man are encountered. Hookworm is one of the most troublesome. Recently I have seen this in a gorilla, giving a pot-belly and marked dyspnoea on exertion which vanished when the worms were eradicated. The condition is troublesome on silver fox farms. Many carnivores and ungulates, especially the pig tribe, harbour ascuids, and most of the larger ungulates harbour strongyles, often in very large numbers. Heavy infestation with threadworms is common also, especially in Primates such as the chimpanzee, *Anthropopithecus troglodytes* the coprophilic habits of which render constant reinfestation inevitable.

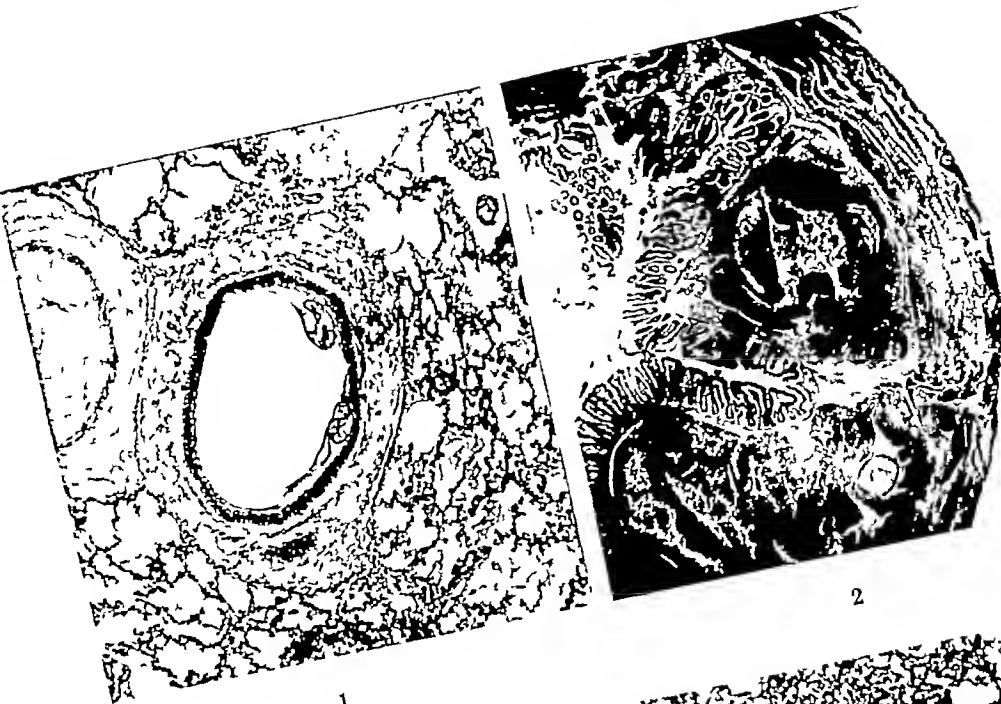
Among reptiles infestation with nematodes is particularly common. I have seen a number of cases of what appeared to be straightforward abscesses in various situations in these animals in the walls of which very small nematodes were found on section.

ARTHROPODA

Small numbers of ticks or lice are found on the skin, hairs or feathers of many animals, but seldom cause trouble and do not appear to act as vectors of disease. The beautifully camouflaged ticks seen round the nostrils, in the axillae or under the tail in large herds, are good examples of these. Some animals always swarm with vermin, a familiar example being the European hedgehog *Erynaeus europaeus*. When animals are ill their ectoparasites often increase greatly in numbers.

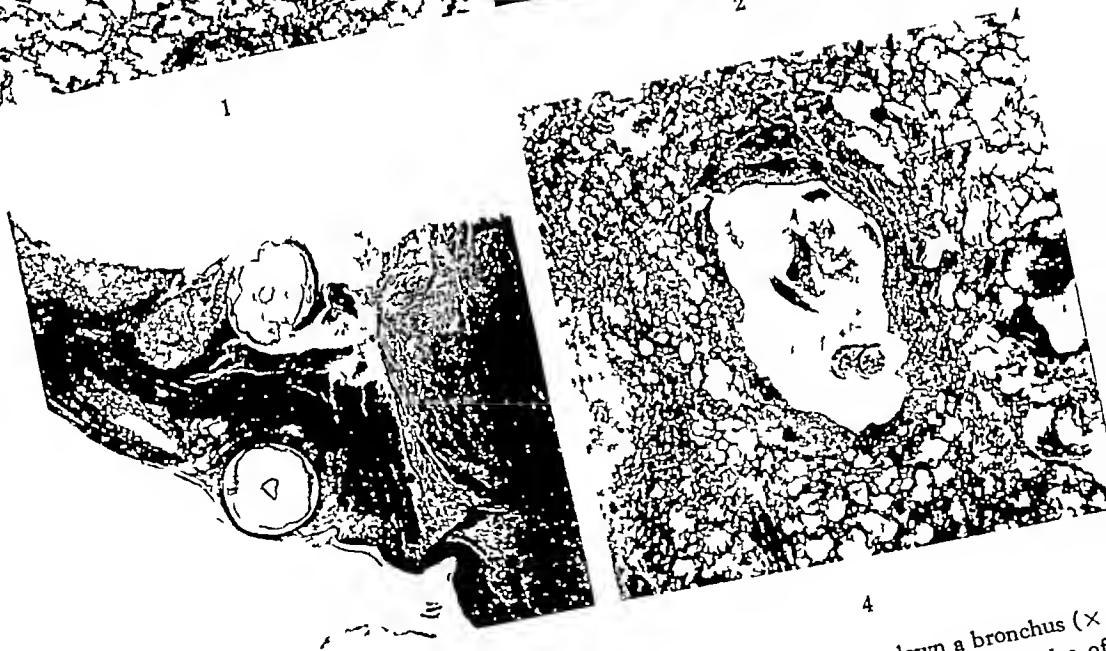
However such parasites can give rise to serious disturbance. Recently we have had an epidemic of severe infestation of the skin in a number of species of snakes with *Ophiomyxus serpentinae*. This appears to kill the hosts, if only by "wearing them down." Mites often occur in the lungs of snakes and may be found in the discharge from their mouths and nostrils. The curious organism *Parocephalus reticulatus* may be found in large numbers in the lungs of some snakes, e.g., pythons. This sometimes infests man.

Sarcoptes and *Demodex* infest the hair of many animals and may be transmitted to man. The most severe case I have seen recently was sarcoptic infestation of the same young gorilla which had hookworm. The animal scratched off almost all its hair and its skin became thickened and rugose over the areas within most easy reach of its hands. Treatment with benzyl benzoate emulsion effected a complete cure. Any trouble with the skin of an animal is noticed at



1

2



3

4

FIG 1—Lung of squirrel monkey, *Saimiris sciurea*, with nematodes passing down a bronchus (\times 17)
 FIG 2—Caecum of Blyth's pheasant, *Tragopan blythi*, in cross section showing nematodes of genus *Heterakis* beneath the mucosa (\times 17)
 FIG 3—Verminous aneurysm from the aorta of a zebra, containing *Strongylus vulgaris* (\times 17)
 FIG 4—Lung of rhesus monkey, *Macaca mulatta*, showing a mite, *Pneumonyssus semicola*, in a bronchus. The epithelium has become denuded and inflammation is starting (\times 17)

R E REWELL

once by its keeper, and he usually institutes vigorous treatment for "mange" for his own protection. Owing to this enthusiasm, the exact diagnosis often remains in doubt.

In mammals, pulmonary acariasis, due to *Pneumonyssus semicola*, is seen most often in macaque monkeys, e.g., the common rhesus monkey, *Macaca mulatta*. The mites settle down in the bronchi where these open into the alveoli. The inflammation and haemorrhage produced obstruct the narrow passages, the alveoli become dilated and so bullae are produced on the surface of the lung. Infestation occurs sporadically and the method of spread is obscure. In monkeys the condition has been known for many years (HAMERTON, 1939), but the diagnosis is made at necropsy and no examination of the blood for eosinophilia appears to have been made yet (Fig 4).

Myiasis is not seen in well-kept captive animals, except for the occasional presence of blowfly maggots in wounds and sores and in the dying "velvet" of the horns of such deer as shed these appendages.

SUMMARY

Diseases of tropical origin in captive wild animals resemble closely many seen in man. However, many conditions familiar in animals in the wild are not encountered. The absence of an intermediate host from the strange environment is an obvious factor limiting the spread of many parasites. However, many conditions not demanding such vectors are not seen either. The high mortality among sick animals on the journey to temperate climates may account for much of this.

Thus, the amount of material available in captive wild animals is small, but its study is of great interest from the general aspect, as well as assisting the understanding of natural infections of man and animals. New hosts for parasites, and the length of time for which some infestations may persist in the absence of an essential vector, are some of the points that can be studied. Individual animals can be observed with a thoroughness that is not often possible in the field.

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DISCUSSION

The President I think you have all enjoyed this talk that Dr REWELL has given us so succinctly and so clearly. It has brought to our minds the great importance of comparative pathology. We have only to refer to the pneumonias disease of monkeys, and call to mind that similar syndromes have been described or suggested as occurring in man, to see the value of studying these diseases as Dr REWELL has done. We have with us Mr H G MAURICE the President of the Zoological Society who is most interested in the Prosectorium, and who has promised to say a few words on this occasion.

Mr H G Maurice The good fortune is mine. I came here this evening on Sir PHILIP MANSION BAIR's invitation which I very readily accepted because I wanted to learn more than I knew about Dr REWELL's work. In the Zoological Society of London we have been very happy in our pathologists. We have had very distinguished men in that office, one of whom, Sir HAROLD SCOTT is here tonight. More recently Colonel HAMERTON retired from the same office. Dr REWELL has not been with us very long, and I have been extremely interested listening tonight, and hearing him give a summary of some of the tropical diseases revealed in captive animals. I have been delighted to find in how short a time he has learned so much. He came into a new field. Captive animals constitute a new field of enquiry and one of our greatest difficulties is to know how to handle the sick animals in the Zoo. We need to get more and more information about the conditions which may be affecting them and it is for that reason that we attach so much importance to the office of pathologist because the pathologist is finding out more and more all the time. We have been very happy in having pathologists—I think we have always had such—who do not merely work in the Prosectorium. They go out, study the animals and watch for sick ones. They look out for the dull eye and staring coat and other such early symptoms. The more they find out the better we shall know how to handle our animal patients and gradually as Dr REWELL has mentioned we are extending our field of knowledge. I did not know until tonight that animals could, as I have understood, carry pathogenic organisms for years and years, where we know they cannot have been renewed through an intermediate animal, because the intermediate animal is not there. There is a vast deal that I do not know about the Zoo, and I am happy to have found out a little more tonight. It was for that purpose that I came here, and not to waste your time.

The President We are very fortunate to have Sir HAROLD SCOTT with us tonight and he has a message for us. There is little he does not know about the diseases of animals in the Zoo. Looking at this lovely array of specimens—most of which were preserved during his tenure of office—we may say that he made the Prosectorium of the Zoo. He has done many good things but that will stand to his credit as one of the greatest he has done.

Sir Harold Scott When our President honoured me with an invitation to take part in this discussion, I wondered whether I could contribute anything useful or interesting. It is just upon 20 years since I ended one of the most fascinating periods of a long career, leaving my post as Pathologist to the Zoological Society of London to take up the appointment of Medical Secretary of the Colonial Medical Research Committee. Detailed records of many hundreds of autopsies I naturally left behind with the Society and my personal duplicate records I handed over to Professor FAIRLEY, who was interested in comparative pathology. In what I have to say tonight I have to rely on stray notes and on reprints of papers which I published during my service at the Zoological Society. If they seem somewhat disjointed, I crave your pardon and indulgence. I shall confine my remarks to diseases common to wild animals and to man, and I would like to say a few words first on the more strictly tropical diseases of man whose analogues occur in captive wild animals. First, then, enteritis and the dysenteries. I have note of a red-faced spider-monkey (*Ateles paniscus*) which died of a liver abscess associated with amoebic dysentery. In the same year there died a white-nosed coati (*Nasua narica*), in the right lobe of the liver there were three separate abscesses, the lowest of which had burst into the peritoneal cavity, setting up general peritonitis. Two years later an orang-utan died of a dysenteric colitis. At the hepatic flexure there were scattered petechiae, but the descending colon and rectum showed an intensely inflamed surface with small crateriform ulcers, with undermined edges, the intervening mucosa being swollen, just as in human intestinal amoebiasis. Of enteritis there were any number of cases. I remember one outbreak among hamadryas baboons on Monkey Hill. I was not allowed by the terms of my appointment to carry out experimental work, so I sent a culture of the organism isolated to Professor LEDINGHAM, who kindly reported that it was *Salmonella aertrycke*, type mutton. Investigation pointed to a carrier among these animals and from it, or others subsequently infected, the disease was spread by contamination of the water or by food pollution and direct contact. In 1927, the same organism was isolated again from the same animals. *Proteus morgani* was the cause of death of several animals. I have notes of its isolation from an orang-utan (*Pongo pygmaeus*), a squirrel monkey (*Saimuris sciurea*), a patas cercopitheque, a green-billed toucan (*Rhamphastos dicolorus*), and others. This, as you know, has been recognized as one of the causes of the summer diarrhoea of infants. An organism of the Flexner group was isolated from a gorilla and from a Canadian beaver. In the latter there were intestinal ulcers and three of these had perforated. Two had been sealed by lymph, but from the third intestinal contents were pouring into the peritoneum and death resulted from peritonitis. Another organism found in human disease but not, I believe, fatal in man, may cause death in animals, that is, *Bact. faecalis alkaligenes*. It produced in them acute enteritis, the mucosa being swollen, deep red, in parts plum-coloured, almost purple, from pylorus to

cloaca. It was isolated from several animals—a *Levillant's* amazon from tortoises, an American plate lizard and a puff adder. An important point is that ulceration was generally absent from these. One had three small ulcers, but these were probably due to the presence of nematodes, and in the puff adder there was a patch of false membrane, 4 to 5 inches above the cloaca. Lastly a reticulated python in December 1927 died from a membranous enteritis. From the intestinal wall and from the bile, *Salmonella paratyphosum* B. was isolated. In man, so far as I know the enteritis produced by this organism is never membranous.

I would like to be able to say something on trypanosomiasis. I thought that it would be of great interest to find out how long animals continued to harbour trypanosomes after their arrival in this country from the tropics. I accordingly planned to examine their blood at intervals, but alas! the idea perished at birth. Sir PETER CHARLES MITCHELL sent for me and while agreeing that scientifically the knowledge would be interesting and it might even be useful, said that the Fellows of the Society would regard the taking of a blood-sneak as vivisection and that a large number of them would immediately resign. Though I ventured to point out that the measure was one of diagnosis I failed to alter his decision. Nay more, I had to give up my vivisection licence at the London School of Hygiene and Tropical Medicine while holding the post of Pathologist to the Zoological Society. To pass to another subject which Dr REWELL has mentioned. In my time at the Zoological Gardens a nocardia or streptothrix disease was the cause of death of not a few wallabies (*Macropus* sp.). It bears a strong resemblance, is in fact closely related to actinomycosis, or lumpy jaw. All zoological societies, so far as I am aware, report cases of this condition. It seems to occur in outbreaks. I looked up the records and found that in 16 years the Society lost 54 out of 180 (30 per cent.) which is about the same proportion as recorded elsewhere. (Sometimes years elapse without any cases.) In about half of them, the jaw is first affected, the teeth are loose, but there is no caries. Infection enters the mucosa at or near the roots of the teeth—in others the base of the tongue is the primary seat the fauces and floor of the mouth being invaded secondarily—occasionally the nasal passages are invaded first. Thence it may extend, as one would expect, along the respiratory or alimentary tract, or both, and to the liver and pancreas. Again, the foot may become involved, perhaps by the animal licking some wound there. Invasion of the tissues is rapid, little, if any barrier of resistance is set up. I will not dwell longer on this. I wrote a special article on it in the Society's *Proceedings* in 1925. Moniliasis, if we except CASTELLANI's pulmonary form as "tea-drinkers' cough," is, in man, a surface infection. In animals, in Batrachia especially it may become very widespread and cause death. I found it spreading in a virulent manner through the viscera, penetrating, destroying and replacing the normal tissues. It caused death of the following Reptilia (under which I include Batrachia)—a corn-snake, a yellow-spotted lizard, bull

frogs, salamanders, tree-frogs, African and Indian toads and a tigrine frog I published a paper on this interesting condition in 1926 *Porocephalus*, a pentastome, a degenerate arachnid (not a worm), is not very common in negroes, cases were recorded in last year's *Tropical Diseases Bulletin* in comment on those of F E STOCK and S L A MANUWA, published in our TRANSACTIONS. Though often symptomless, *Porocephalus* may cause serious illness, as collapse of lung and, indirectly, intestinal obstruction. The adult form I found quite frequently in snakes, pythons and vipers, and the nymphal forms in lions, leopards, mandrill and other animals.

I am loath to take up more of your time but, if I may, I would like just to touch upon, not discuss, some of the many conditions which are common to man and wild animals. They have all been culled from my work at the Zoological Society's Prosectorium —

(1) Intestinal obstruction was fairly common, especially intussusception, one was very unusual and was preserved for the School Museum. In a serval the upper part of the duodenum had passed through the pylorus into the stomach and was strangulated. Diaphragmatic hernia was another unusual form, it occurred in a tiger cat (*Felis aurata*) and a lion cub, much of the abdominal contents had passed up into the thorax.

(2) Intestinal perforation, often by ulcer, sometimes by nematodes, sometimes by a foreign body. A tin-opener perforated the gizzard of an ostrich and penetrated the portal vein. The digestion of an ostrich is proverbial, but the following must surely be a record. What I removed was mounted and preserved in the Museum. There were three handkerchiefs, three gloves, 3 ft of cord, two iron-wire staples complete and several fragments, a 4-inch nail, four halfpennies, two farthings, a franc, a motor-tyre valve, part of a magneto spanner, a collar stud, a lead pencil, a picture hanger, an alarm-clock winder, a watch swivel, a fragment of a locket-chain, a wooden film-roll, a screw and sundry small pieces of metal which had been snipped off from the wire enclosure.

(3) Cardiovascular. Aneurysm of the heart in a hooded vulture and a common rat (*Rattus rattus*), of the aorta in a Malayan palm civet, in a walrus, in a falcated teal and in a scarlet tanager (*Rhamphocelus bresilius*). Rupture of the heart occurred in an American tree-sparrow (truly this died of a broken heart, perhaps there was rumour of its having to leave its English bride). Congenital heart defects occurred in a *Hemitragus jemlaicus* and a De Brun's wallaby. V D H occurred in various forms.

(4) Neoplasms, particularly malignant neoplasms, were far from uncommon. I have seen sarcoma of heart, liver and brain in a porose crocodile, another of the heart in an Indian rhinoceros, extensive endothelioma in a brindled gnu, the abdomen was enlarged by the growth and, in fact, the animal had been

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(3) Cardiovascular. Aneurysm of the heart in a hooded vulture and a common rat (*Rattus rattus*), of the aorta in a Malayan palm civet, in a walrus, in a falcated teal and in a scarlet tanager (*Rhamphocelus bresilius*). Rupture of the heart occurred in an American tree-sparrow (truly this died of a broken heart, perhaps there was rumour of its having to leave its English bride). Congenital heart defects occurred in a *Hemitragus jemlaicus* and a De Brun's wallaby. V D H occurred in various forms.

(4) Neoplasms, particularly malignant neoplasms, were far from uncommon. I have seen sarcoma of heart, liver and brain in a porose crocodile, another of the heart in an Indian rhinoceros, extensive endothelioma in a brindled gnu, the abdomen was enlarged by the growth and, in fact, the animal had been

purchased originally because it was believed to be in what is called euphemistically an interesting condition and there was a chance of acquiring an additional animal for the Gardens, two for three-halfpence as it were. In very truth on this occasion they bought a pig in a poke. It was indeed interesting but only pathologically. Sarcoma of the thyroid and carcinoma of the parathyroid in an otter (*Lutra lutra*) and squamous epithelioma of the tonsil in the wolf. Thyroid tumours are common, I cannot say why in raccoons and foxes. Other conditions I may mention are hæmorrhagic pancreatitis, duodenal ulcer in lion cub and a feline genet (certainly not in these instances due to smoking) congenital malformations of the kidney in reptiles, birds and mammals hydatid and coenurus osteitis fibrosa in a pigmy elephant (the skull cut like cheese), and lymphocytic leucæmia in a Senegal chameleon (white red corpuscles 5-3). Of these Dr REWELL mentions coenurus in the brains of ungulates. I have put on record two cases of coenurus in another family of the greatest interest. Both were in Gelada baboons (*Theropithecus gelada*) and were seen in the same year. In one the masses were very large. There was one the size of a coconut in the right breast another as large as an orange at the right shoulder a third, the size of a cricket ball at the back of the right upper arm a fourth the size of a five shilling ball, or should I say a golf ball, in the submental region, and there were smaller ones in the mediastinum and pericardium. The thymus was a cystic mass of them and there was yet another the size of that in the submental region in the right perirenal tissue. This animal had been in the Gardens 18 months and a swelling of the right breast had been noticed 6 to 7 months later. The second was the subject of fewer and smaller tumours but was even more interesting. Increasing weakness leading gradually to paralysis of the hind limbs had been noticed for 3 to 4 months. The condition was definitely spastic in character and sensation was defective also latterly. This animal had been in the Gardens for 16 months. At the autopsy three small cysts were seen low down in the left pleural cavity. One of these had penetrated through the intercostal spaces near the spinal column and had invaded the vertebral canal and gradually compressed the spinal cord in the lower dorsal region. In conclusion, may I relate briefly a sad love-story of the Zoo? Love is a disease common to man and animals, and in the tropics as in temperate climates. A female hamadryas baboon, the widow of one known as Murphy was wooed on *secondes noces* by four suitors simultaneously. A battle-royal ensued in which the lady suffered somewhat, and one of the gentlemen, having gained a temporary advantage, fled with her into the water whither his rivals followed to renew the fight. When the final victor emerged, bringing with him his prospective and hard-won bride, she was found to be dead. After boding a wake (may I remind you she was Mrs. Murphy?), he and his companions the next morning, presumably to divert suspicion, returned the body to the pond. At the inquest I found several bruises and scratches but not sufficient to account for death, which was definitely due to drowning. The successful

DISCUSSION

sutor had retained his hold on the prize, keeping her under while he fought for her, adopting the motto of a well-known English family, "*Quod habeo, teneo*" ("What I have I hold") He held it a little too long.

The President We knew we were going to get something good, and we have We are a very animal-minded race in these islands Everybody loves the Zoo, and I hope this spate of humanitarianism which is surging through the present Government may not be extended to the Zoological Gardens I have talked to a good many people, who think the next piece of legislation should be their abolition as being cruel to animals Sir HAROLD SCOTT spoke about the hamadryas baboon During one heat wave several years before the war a very kind old lady, watching the monkeys on Monkey Hill, observed that their posteriors had become overheated from sitting on the rocks She wrote at once to Sir PETER CHALMERS-MITCHELL, pointing this out, and suggesting that they should be provided with air cushions so that they should be prevented from being sunburnt on this particular part

Professor Edward Hindle With reference to the comments by Sir HAROLD SCOTT and other speakers, there are one or two points to which I should like to call attention In the first place, I think that many people have quite a wrong idea about the wealth of pathological material available in zoological gardens You have to remember the history of most of the animals before they arrive at a zoo With few exceptions, they have all been in captivity under varying conditions, often unfavourable, for some considerable time, and in the case of all but our few native animals, have had to undergo a trying sea journey in addition It must be obvious that any animal with anything in the nature of an acute infection would succumb en route The only exotic infections, therefore, that one meets are those of a chronic nature, such as plasmodia, haemogregarines, helminths, etc., and possibly mycosis, or even some bacterial infections, though of this we have no definite proof To be sure, there are occasional neoplasms, but these are essentially the same as those studied in laboratory animals

The diseases of animals in zoos cannot be regarded as in any way giving a true picture of what occurs in nature We know but little about this subject, but wild animals must be exposed to a variety of infections which never reach zoological gardens One only needs to consider the number of virus infections that have come to light by the investigation of recently collected animals from tropical forests, especially in connection with the study of the epidemiology of yellow fever

Bearing in mind these and other difficulties, it is all the more creditable that so much has been accomplished in the Zoological Society's Prosectorium and the paper by Dr REWELL this evening ably summarizes some of these discoveries You will notice that the number of protozoal infections, even ov-

a long period, is very small, understandably so, for one could only expect to see chronic infections which had relapsed. With regard to deficiency diseases animals live under such very diverse conditions and in many cases it is extremely difficult to decide on any particular diet. As a rule, however, animals have been tested out to some extent before reaching the Zoo, and have become accustomed to whatever diet has been available in their captivity and during the journey. It is surprising what unnatural diets may be successful. For example anteaters which in nature feed almost entirely on ants, do well on a very artificial mixture of minced meat, eggs, milk, and so-called ants—eggs, really pupae. In fact, we had a disaster last year in trying to give an anteater its natural food. An ants' nest was brought in from Whipsnade and given to two young tamanduas. These animals feed on tree ants which they obtain in almost pure culture by pulling off the bark of infested trees. When given a pailful of ants' nest they readily licked up the ants and pupae, but in addition swallowed so much earth that they died of intestinal obstruction.

Occasionally we meet with diet deficiency in the literal sense of the word by the animal not having been given sufficient food. Recently an interesting animal reached the Gardens after a long sea journey and on arrival was very savage. There was good reason for this as the animal, about the size of a large cat, had been given only one banana a day for food and was virtually starving. After a few days on a more adequate diet its disposition completely changed, and it now plays with the keeper and follows him like a dog. There is also a record of a pair of young caracals that had been fed exclusively on peanuts during a journey from Gambia to London, and yet one of them survived!

Deficiency diseases present a very real problem, however, and little is known about the conditions governing the nutrition of all cold-blooded animals. For example chameleons, whose natural diet is almost certainly insects, never last very long in captivity although fed on what would seem to be a natural diet.

Finally I should like to refer to one difficulty which confronts every pathologist working at the London Zoological Gardens. It has been considered necessary to avoid doing anything which might be interpreted as vivisection in the strict sense of the term, and therefore there has been no periodic examination of the blood of animals, which might bring to light various latent infections and assist in the treatment of disease in its early stage. I need only refer to recent articles in the Press concerning the giant panda—"weeping for the forests of Szechwan, or shading its eyes from the dazzling sunshine of Regent Park," coupled with the demand that it should be sent back to China, to realize the sort of criticism an institution like ours has to face.

In view of the difficulties, therefore, it is all the more creditable that so much has been accomplished by the study of animals after they have died, and finally I should like to join in saying how happy we are in our new Zoo pathologist.

Dr J Ungar I can say little with regard to the diseases of animals in captivity. In the research laboratories we are trying to keep healthy stock of small laboratory animals for chemotherapeutic and immunological studies. We aim at the eradication of spontaneous diseases in laboratory animals, e.g., *Salmonella* in mice, which are seldom a source of infection to the attending staff. In this connection, I would like to mention the newly established Laboratory Animals' Bureau, under Dr GLOVER, which will fulfil a very useful function in keeping a register of different strains of laboratory animals suitable for experimental work in this country. How important it is, for chemotherapeutic work, to keep different lines of small animals, can be seen in the two examples I quote —

Some years ago we started work on experimental pertussis. We tried to induce an artificial infection in a number of small animals and we found that only two strains of mice were susceptible to intra-nasal infection with a virulent suspension of *Haemophilus pertussis*.

The second example is experimental work in tuberculosis. Guinea-pigs and rabbits are susceptible to different strains of *Mycobacterium tuberculosis*. It is essential to have small animals in which we can induce infections in a shorter time than guinea-pigs. Although the pathological changes are quite different in mice from lesions induced in guinea-pigs, they can be used in larger numbers to study chemotherapy of tuberculosis. One strain of mice proved to be more susceptible to the experimental infection with *Mycobacterium tuberculosis* than others.

In the selection of pure lines we have the help of geneticists, and I think that the co-operation of veterinary surgeons and pharmacologists will help to maintain different strains of small animals in a way which will enable experimental studies. I am sure that public opinion will not object to its being done.

Dr C A Hoare In connection with Sir HAROLD SCOTT's remarks about the difficulty he experienced in the examination of animals for the presence of trypanosomes, it may be recalled that recently Colonel HAMERTON reported in the *Proceedings of the Zoological Society* (1947, 106, 611), the finding of *Trypanosoma congolense* in an African antelope (*Tragelaphus* sp.) which had been in the Gardens for about 12 years. It is obvious in this case that the infection had been contracted in Africa. This finding raises an interesting question since some of the mammalian trypanosomes are readily transmitted mechanically by various blood-sucking Diptera, it is conceivable that under suitable conditions a trypanosome infection imported into this country with wild animals might spread to other inmates of the Zoological Gardens, especially to ungulates.

Dr C M Wenyon Dr REWELL mentioned my examination of blood films of animals that had died in the Zoo. I do not know how many years I have been examining these, but I think about 30. I have found the usual organisms—species of *Plasmodium*, *Haemoproteus*, *Trypanosoma*, *Haemogregarina*, and other parasites. In the pigmented parasites of birds and reptiles I have

always recorded them as *Plasmodium* or *Haemoproteus*. My reason for calling a parasite *Plasmodium* is that in the peripheral blood I have been able to find schizonts, i.e., reproduction forms, and I call the parasites *Haemoproteus* when there are no reproducing forms. That separation is fairly accurate though in the case of the parasites I call *Haemoproteus* one might occasionally find reproducing forms if a longer search of the film were made. As regards *Plasmodium* in birds in the Zoo, it seems to me that they may be infected there. It is quite possible that mosquitoes in the Zoo may get infected from one bird and inoculate the infection unto others, so that the *Plasmodium* in that way may be transmitted to hosts which are not the natural ones. Penguins commonly have *Plasmodium* and sometimes the infection is so great that one is inclined to think the death is caused by it. The *Plasmodium* of penguins is fairly well known and seems to be peculiar to the penguin itself. Dr HOARE mentioned the case of *Trypanosoma congolense* which occurred in an antelope (*Tragelaphus gratus*) from West Africa. That infection was found by myself in the animal, which had died from some renal condition after being in the Zoo about 11 years. There was no evidence that it had contracted the infection from any other animal in the Zoo and the observation shows that antelope may carry a trypanosome infection for many years.

The President. It has always puzzled me how these penguins get injected with plasmodia. They have come from South Georgia, where there are no mosquitoes to transmit it. Do they become infected on the voyage? One particular bird, a king penguin, from ice-bound South Georgia, was supposed to be infected at Buenos Ayres on its way to this country.

Dr Carmichael Low. I have always maintained that the Zoological Gardens are a gold mine for anyone wishing to study comparative pathology and research. If one considers that all these animals, and especially birds, that have micro-filariae in their blood must also carry the adult forms, and require an intermediate host to carry the infection to others, then you will understand that a person might spend the whole of his life studying this group alone. There is room for several pathologists really to carry out research on the vast amount of material available. You have heard from others, Sir HAROLD SCOTT especially of the pathology you find in animals, tumours, bacterial infection, etc. I was the physician to the gorilla in which he found the Flexner's bacillus, and I remember when it was dying discussing with Professor WOOLMOND the possible cause of its decline. He thought a vitamin deficiency but I said No, more likely tubercle. Sir HAROLD did an autopsy and found that it died of bacillary dysentery from which he isolated a Flexner bacillus.

There is a great field in comparative pathology as a help for human diseases,

DISCUSSION

and I hope Dr REWELL will carry on with the valuable work he is doing
congratulate him on his paper

Air-Cdr T C Morton I would like to congratulate the speaker on a most interesting address

I was particularly interested in the specimens and remarks on acariasis producing such marked pulmonary lesions in monkeys I have been interested for some years in Weingarten's tropical eosinophilia and have always felt rather dubious as to the alleged role of mites in this condition, as suggested by CARTER and others Stovarsol and other arsenicals relieve Weingarten's syndrome in human beings, it would be well worth while to see if these drugs prove to be curative in acariasis of the lungs in monkeys, if they do, the case for mites being responsible for Weingarten's tropical eosinophilia would be strengthened

Dr R E Rewell (in reply) I must re-emphasize my opening remarks, and thank Dr HINDLE for doing so as well A zoo gets only fit animals Those that are ill die on the journey or in quarantine A high proportion die within 6 months of arrival, so that the few fit ones alone live for any length of time Another limitation is the small number of any one species under observation In certain monkeys, a dozen individuals may be seen at one time and there may be half-a-dozen chimpanzees, but this is all, so that the detailed pathology of any one species can never be worked out by one observer Any abnormal conditions found in the three specimens of *Tarsus* that have been brought to England recently are likely to remain as isolated observations and never to be followed up

To Sir HAROLD SCOTT I must report, however, that a new type of foreign body is now seen in the gizzard of the ostrich The service cap-badge appears to have an irresistible attraction for this bird.

I am glad that the case of trypanosomiasis in *Tragelaphus gratus* was discussed again The fact that only one case has occurred in 30 years shows again how rare such things are in captive animals Do not forget that my tables showing cases of blood infestations covered as long a period as 15 years

The outbreak of *Salmonella aertryke* infection observed by Sir HAROLD SCOTT, and the very few similar ones observed in other Primates, show also how seldom such things are seen Like Sir HAROLD, I have found *Proteus morgani* in the faeces and intestines of animals with enteritis, but I do not believe it to be a true pathogen In the cases of dysentery which I mentioned *P. morgani* was a contaminant and was eliminated only by prolonged culture on desoxycholate-citrate agar It is now well known that this organism more easily outgrow the dysentery organisms and its colonies be indistinguishable

from them after only 18 hours on this medium. In the absence of experimental verification, it is very difficult to be certain of the pathogenicity of any organ sm isolated at necropsy or in the living, unless this is identical with a pathogen producing the same lesion in another species.

South Georgia is not within the Antarctic Circle. I looked this up in the map recently in connection with this problem of the presence of *Plasmodium* in penguins.

In my talk I was able to deal with selected points only out of a wealth of material accumulated despite the limitations mentioned. I am glad that the selection appears to have included so much of interest to this Society

COMMUNICATIONS.

INVESTIGATIONS ON THE ANTIBILHARZIAL ACTION OF MIRACIL D (NILODIN)

BY

J M WATSON, D SC (LOND), A R C S, *
Senior Helminthologist, Wellcome Laboratories of Tropical Medicine, London,
M ABDEL AZIM, D M S (CAIRO), D T M & H (ENG),
Director, Bilharzia Snail Destruction Section, Ministry of Public Health, Cairo,

AND

A HALAWANI, M D (CAIRO), D T M & D T H (LIV)
Director, Fouad I Research Institute and Endemic Diseases Hospital, Cairo
(From the Ministry of Public Health, Cairo)

INTRODUCTION

A satisfactory drug for the oral treatment of bilharzia disease has been the object of research almost since the discovery of the parasite itself. Prior to the

* We wish to express our gratitude to His Excellency, Dr A T SHOUSHA PASHA, Under-Secretary of State for Medical Affairs, Ministry of Public Health, Cairo, for the assistance and encouragement with which he supported us during the carrying out of this work. We are also indebted to the Director and staff of the Veterinary Serum Institute at Abassiah (Ministry of Agriculture) for assistance in the maintenance of experimental animals. To Brigadier J S K BOYD and Dr C H BARLOW we owe thanks for help and advice, and we would also like to thank those of our colleagues, both in the Ministry of Public Health and elsewhere, who so willingly assisted us in the clinical work and in the carrying out of the biochemical and pharmacological tests, notably Dr HAFIZ, Dr ABDALLAH, Dr AWNI and Dr AYADI, of the Research Institute, Dr AMEEN, of the Oosim District Hospital, Dr NEWSOME and Dr WOOTTON, of the Bilharzia Research Unit of the British Medical Research Council, to Dr P B MARSHALL, Mr L G GOODWIN, Mr O D STANDEN and Mr R BROMFIELD, of the Wellcome Laboratories of Tropical Medicine and to Dr CUTTING and Dr RADCLIFFE, of the C M S Hospital in Old Cairo.

The miracil D (or nilodin) used in the carrying out of the present work was supplied by the Wellcome Foundation, Limited. The drug was made available in three forms, namely, a finely divided powder for use in the animal experiments, and both plain and enteric-coated tablets for use in the clinical trials.

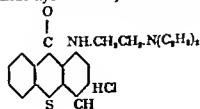
introduction of treatment by intravenous injection of tartar emetic, extract of male fern was recommended (MANSON 1906), but re-investigation by RICHTER (1936) has shown this drug to be ineffective. Thymol benzene was used with apparent success by EKES (1915) and ROBERTSON (1916). GORDON (1926) and OWEN (1928) investigated the use of emetine periodide while GORDON and HICKS (1930) employed auremetine these treatments were apparently effective but were prohibitive in cost. Later the use of acriflavine derivatives enjoyed a vogue and favourable results were claimed by FISHER (1934) but FAKIRY (1934) KHALIL and SALAH (1934) and GIOVANNOLA (1936) denied these claims. A copper salt, cuproquin or paludex (sodium cupro-oxyquinolin sulphate) was later introduced by VAN NITEN (1937) and SERRA (1937) who believed it to be effective, but CAWSTON (1938) and DAVID (1938) concluded that cuproquin treatment was a failure. KHALIL and SALAH (1934) found atabrin (mepacrine) to be ineffective, while CAWSTON (1936) came to the same conclusion with regard to carbon tetrachloride.

Various attempts have been made to administer antimony by mouth. Thus tartar emetic was administered by the oral route by WALKER (1928). GONZALEZ RINCONES (1945) administered enteric-coated tablets of tartar emetic combined with atropine and vitamin B₁ while AYADI (1947) treated experimentally infected monkeys by oral administration of reprodral combined with riboflavin. Despite the strong emetic action of the drug, considerable success has been claimed by these workers.

During the last decade research workers of the I G Farbenindustrie at Elberfeld have synthesized and tested a number of organic compounds for bilharzicidal activity. One of their series, developed originally by MADSS was found to contain several active compounds, the most promising of which was termed miracil D. Although satisfactory results were obtained in animal experiments by HAKUTH and GONNERT (1945), no facilities then existed for clinical trials of the new drug. Investigations were therefore undertaken by the present authors to verify its bilharzicidal action and toxicity both in laboratory experiments and clinical trials, in view of the advantages which a satisfactory oral treatment promises to yield.

HISTORY OF MIRACIL D

Miracil D which is a thioxanthone derivative (1-methyl-4-diethylaminoethoxythioxanthone hydrochloride) has the following structural formula —



It is a fine yellow powder, moderately soluble in water, a 2 per cent solution being obtainable at room temperature

KIKUTH and GONNERT* claimed that it showed high activity against *Bilharzia mansoni* in mice and monkeys, as little as two doses of 5 mg per kg at 3 days' interval being sufficient to produce a cure in the latter species. An equally favourable result was stated by KIKUTH* to have been achieved by VOGEL, working in the Institute for Tropical Disease, Hamburg, in animals infected with *B. haematobia*, but no details or confirmation of this claim are available. Nor does KIKUTH himself appear to have made any tests on animals infected with *B. haematobia* although it is known that a strain of this species was being maintained in his laboratory.

The toxicity of the new drug was claimed by KIKUTH to be low, the chemotherapeutic ratio in mice being 1:30, whereas that of foudadin lies between 1:2 and 1:4. Another German worker, HECHT,* made a preliminary investigation of the pharmacology of miracil D, which indicated that it was, perhaps, more toxic than had been originally supposed.

According to KIKUTH,* the adult parasites only succumb slowly to the effects of the drug, still living but severely injured worms being found in the liver several weeks after the administration of curative doses. The principal action appeared to be on the reproductive organs, the production of spermatozoa by the male and of yolk and egg-cells by the female having already ceased a few days after treatment. No diminution of fertility in mammals resulted from the regular administration of the drug.

Immature worms were claimed by KIKUTH* to be unaffected by miracil D, in doses far exceeding those found to be lethal to the adult parasites.

More recent work has somewhat modified the original picture of the new chemotherapeutic agent. Its pharmacology was re-investigated by WOOD (1947), whose results tend, in general, to show that it is less toxic than the work of HECHT had indicated, and further experiments carried out by workers in the Wellcome Laboratories of Tropical Medicine confirmed WOOD's findings.

Methods for the estimation of miracil D in the body fluids have been described by LATNER, COXON and KING (1947), while HALAWANI, NEWSOME and WOOTTON (1947) have recently published an account of the investigation of the concentration of the drug in the blood at different time intervals after administration of various dose levels, and have shown that a correlation exists between the blood-level of the drug and the urea clearance rate.

* Accounts of the work of KIKUTH, GONNERT, HECHT and the other original German investigators may be found in the Reports of the Combined Intelligence Objectives Sub-committee (File XXV, 54).

A full account of the work briefly recorded in this paper has been published as an official *Ministry of Public Health Report* from the Government Press, Cairo.

Clinical trials carried out by BLAIR, HAWKINS and ROSS (1947) in the Southern Rhodesian Public Health Department produced disappointing results, both in patients infected with *B. haematodes* and those infected with *B. manson* only 22 per cent. of the former and 18 per cent. of the latter showing apparent cures 8 weeks after the end of treatment. There is reason to believe however that the dose level used in these trials was too low in relation to the urea clearance rate to maintain the necessary minimum blood-level of the drug to kill the worms.

Workers in America are also believed to have obtained disappointing results but no published details are as yet available.

TOXICITY

It may not be out of place to recapitulate briefly the salient facts about the toxicity of miracil D which have emerged from the work of HECHT (1943) and WOOD (1947) and have been confirmed by investigators in the Wellcome Laboratories of Tropical Medicine in connection with the present work, before proceeding to relate our own observations on the effects in man.

With regard to the acute toxicity sub-lethal doses of the drug caused a fall in blood pressure and a slight augmentation of respiration. Simultaneous administration of adrenalin reduces the drop in blood pressure but produces a greater stimulation of respiration than can be attributed to either drug alone. Since the depressor effect is still observed after administration of atropine it may be concluded that nikodin acts directly on the heart and blood vessels rather than on the nervous mechanism. In fetal dosage death is due to the direct effect on the heart which stops before respiration. Above a certain level intravenous injection causes convulsions similar to those produced by picrotoxin. According to WOOD miracil D produces a mild spasmolytic action on intestinal muscle but respiration and contraction of skeletal muscle are unaffected.

The lethal dose varies according to the species of experimental animal and the route of administration. In mice, the average lethal dose (L. D.₅₀) lies between 20 and 40 mg per kg when the drug is given intravenously and between 140 and 200 mg per kg following intraperitoneal injection but in the case of subcutaneous injection death does not follow the largest dose which can be conveniently administered (500 mg per kg), while oral administration does not cause death in even higher doses than this. The lower dosage is tolerated in repeated administration, 125 mg per kg orally being supported for 10 days but subsequently causing mortality which reaches 100 per cent. after 20 days. No mortality followed a daily dose of 67.5 mg per kg after 23 days. Guinea-pigs showed similar reactions but rabbits were more sensitive and in the case of cats and monkeys the lethal dose by the oral route could not be ascertained since the drug was vomited.

marked by extreme lassitude with intervals of restlessness, insomnia, nausea, metallic taste, tingling of the skin, headache and lumbar pain.

No contra-indications have as yet been determined but it is probably inadvisable to administer the drug to patients with organic disease of the heart, liver or lungs.

ANIMAL EXPERIMENTS.

1. TECHNIQUE.

Mice, jerbils and monkeys were employed as experimental animals. Infection was carried out in all cases by the cutaneous route, each animal in a group being exposed individually to the same depth and volume of water containing the same number cercariae since it has been shown (ASDELL, ALLEN and WATSON 1948) that this is the most satisfactory method of establishing a uniform infection with bilharzia in experimental animals. Despite every care in the maintenance of the animals on a balanced diet and spacious cages kept in cool enclosure, considerable mortality occurred among the small animals during the period of 1 or 3 months which elapsed before the worms reach maturity, no experiment being undertaken until all the surviving animals in the group had been proved to be passing viable ova. In consequence although each group contained twenty-five animals at the commencement the number surviving to the end of the experiment was considerably lower.

Oral administration of the drug was achieved by the usual method of syringe or stomach tube except in the case of the monkeys which received enteric-coated tablets such as were used in the clinical trials. The dosage was calculated on the basis of the average weight of the animals in each group. Each animal was weighed at autopsy and that the exact dosage administered might be calculated since in practice some animals weighed more and others less than the estimated mean. It having been found impracticable to weigh each animal before treatment, the source of error inherent in the possibility that the weight of the animal might change between administration of the drug and autopsy was perforce ignored.

In view of KRAUTH's statement that miracil D was slow in producing its lethal effect on the worms the treated animals were allowed to remain for a period of approximately 1 month before autopsy. Surviving individuals in each group were then killed and careful postmortem examination conducted in order to determine the number, situation and condition of the parasites and their ova. Any adult worms found were immediately transferred to normal saline at 37° C. Since worms removed from untreated mice immediately after death were actively motile in this medium, complete absence of movement on the part of the parasite was taken as the criterion of death, and was confirmed by turbid appearance of the tissues when examined under high magnification. Normal appearance and movement in flame cells were taken as sufficient evidence of the viability of ova, especially when ciliary movement was apparent after hatching by pressure. The contingency that non-viable ova alone were apparent in the liver or wall of the gut, a small portion of tissue was teased in filtered Nile water which was maintained at 37° C. for 24 hours in order to observe whether any hatching took place.

Those animals which died after the beginning of treatment, but before the time came for them to be killed, were also autopsied. Naturally less reliance can be placed on the results obtained in such cases, since several hours sometimes elapsed between death of the animal and its discovery and examination. However tests showed that in untreated animals the parasites were still alive and actively motile many hours after the death of the host—sometimes after preservation of the corpse over night by refrigeration.

In the case of the monkeys the faeces were regularly examined for ova but no attempt was made to assess the progress of cure in the smaller animals by this method, since even in untreated mice the percentage of degenerate ova was high and the total egg-output too small and erratic on which to base any valid conclusions.



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II EFFECT OF MIRACIL D ON ADULT WORMS

(a) *Bilharzia mansoni*

Six groups of mice which had been infected with *B. mansoni* were treated with miracil D commencing about 60 days after infection. In the first four groups treatment was given at 3-day intervals, the dosage rate being as follows —

- Group 1 Received two doses of 5 mg per kg
- Group 2 Received two doses of 10 mg per kg
- Group 3 Received four doses of 5 mg per kg
- Group 4 Received four doses of 10 mg per kg.

Living adult worms were recovered from the animals in Groups 1, 2 and 3, but those recovered from the animals in Group 4 were dead. Moreover, while viable ova which yielded living miracidia were found in the faeces and gut-wall of the mice in the first three groups, only non-viable and degenerate ova were recovered from the animals in Group 4, and no hatching took place. Thus the minimum total dosage of the drug that appeared to have a lethal effect upon the worms was 40 mg per kg.

In the 5th group each animal received three doses of 25 mg per kg, given on alternate days. All but one of the animals in this group died or was killed before the lapse of 3 weeks from the end of treatment and living active worms were recovered from each one. The remaining mouse was killed and examined on the 39th day after treatment, all the worms which it contained were dead. In the sixth group each animal received five doses of 25 mg per kg administered over a period of a fortnight. Of the eight animals in this group, two died during treatment and three during the following 3 days, the remaining three were killed, one 28 days and the other two 33 days after the end of treatment. The autopsies showed a progressive lethal effect on the parasites, as may be seen by reference to the table, the majority of the worms being dead in those animals which survived to the end of the experiment. Thus viable ova were found in each case, which may be explained by the fact that a few parasites survived the action of the drug. It is not without significance that in all cases, even those in which insufficient drug to have a lethal effect had been administered, the worms had retreated from the mesenteric veins and the distal part of the portal vein into its proximal part and into the liver, an effect which is characteristic of drugs that bother the worms. The significance of this observation is emphasized by comparison with the state of affairs in mice which had received no treatment, in which numbers of worms were found in the mesenteric veins in every case.

Two groups of *mansoni*-infected jerbils were treated with miracil D. In the first of these groups, four doses of 25 mg per kg were administered on alternate days, starting on the 35th day after infection. Living active worms and viable ova were found in large numbers in an animal which was killed the day before treatment, in one which died during treatment and in one which died immediately following treatment. The remaining animals were killed and autopsied 22 days after the end of treatment when it was found that most of the worms had retreated into the liver and that most but not all of them were dead. Viable ova were found in the faeces and rectal wall.

In the second group of jerbils, five doses of 25 mg per kg of the drug were administered, starting on the 77th day after infection. Two of these animals were killed and examined 21 days after the end of treatment, when living adult worms and viable ova were found together with dead and disintegrating worms in the liver. The remaining animals were therefore given a second treatment consisting of six doses of 50 mg per kg administered on successive days. These individuals were autopsied 41 days after the end of the second treatment. In all of them worms were absent from the mesenteric and portal veins but numerous dead, disintegrating and encapsulating specimens were found in the liver, and sections showed that encapsulation had taken place and phagocytosis was proceeding actively. Small numbers of blackened and degenerate ova occurred in the walls of the large intestine and rectum.

(b) *Bithersia harmatobia*.

Three groups of mice which had been infected with *B. harmatobia* were treated with miracil D commencing about 100 days after infection.

I the first group which received four doses of 10 mg. per kg. on alternate days, not only those mice which died soon after treatment but also those which survived to be killed and autopsied on the 38th day after receiving the last dose were all found to contain living active worms and to be passing viable ova.

In the second group which received six doses of 10 mg. per kg. on successive days, one mouse was autopsied on the 28th day after the end of treatment, when it was found that all the worms had retreated to the liver but that they were still alive and active. I the remaining animals which were killed and examined on the 38th day after the end of treatment, the worms were found to have re-emerged from the liver but most of them were dead and all the ova observed were degenerate.

In the third group which received four doses of .5 mg. per kg. on alternate days, all the animals were killed and autopsied on the 38th day after the end of treatment. I 40 per cent. of this group degenerate ova and dead worms only were found, while in the remainder viable ova and living active worms were observed to be present.

I addition to the mice two monkeys were heavily infected with *B. harmatobia*. Both of these animals developed severe bilharzial dysentery the faeces containing much blood and mucus and abundant ova. Both animals received five doses of 250 mg. I the case of monkey No. 1 the drug was administered on alternate days and the dose-rate worked out at 33 mg. per kg., whereas in the case of monkey No. 2 the drug was administered on successive days and the dose-rate worked out at 40 mg. per kg. During and following treatment the animals were lethargic, ate no food except green vegetables, and balanced with difficulty giving the impression that they were giddy the nose and faeces took on distinctive yellow colour. Blood and mucus gradually disappeared from the stools, which were once more entirely normal by the time a week had elapsed from the last dose while ova disappeared by the 3rd day in monkey No. 1 and the 10th day in monkey No. 2. Moreover the faeces remained consistently negative for ova, no relapse taking place. Monkey No. 1 was killed and autopsied 3 months after the end of treatment when no worms or ova were found anywhere in the body while macroscopically all organs appeared normal.

III. EFFECT OF MIRACIL D ON IMMATURE WORMS.

A single batch of mice all of which had been infected with *B. muris* on the same date were divided into four groups, and treatment consisting of four doses of 10 mg. per kg. at 3-day intervals was administered commencing respectively on the 8th, 15th, 22nd and 29th days after infection. All those mice which survived were killed and examined on the 55th day after infection when living adult worms were found in the mesenteric and portal veins as well as in the liver in every case while viable ova from which hatching took place were recovered from the faeces. In this dosage and under these conditions miracil D evidently has no effect on the immature worms. However it is possible that higher dosage level might have been effective and it is on intention to carry out further experiments to elucidate this point.

IV. INFECTED MICE.

I order to provide standard for comparison for the treated animal to further groups of mice were infected, one with *B. muris* and the other with *B. harmatobia*. These animals were killed and examined when the passage of viable ova in the faeces indicated that the parasites had reached maturity. Living adult worms were found in the mesenteric

B. harmatobia takes considerably longer to mature in experimental animals (9) to 100 days) than *B. muris* (about 50 days)

CLINICAL TRIALS.

For the purpose of administration to human patients, enteric coated and plain tablets of two sizes, one containing 200 mg. and the other 50 mg. of the drug were used. The enteric coating, the advisability of which had been suggested by the fact that vomiting had followed administration to cats and monkeys (HICHT 1945), was later found to be unnecessary and while the coated tablets were employed throughout the trials recorded below plain tablets were successfully used in some of the later work (as yet unpublished). BLAIR, HAWKING and ROSS (1947) used plain tablets without ill-effects in their Rhodesian trials. In the preliminary trials, which were carried out in part at the Endemic Diseases Hospital and in part at the Church Missionary Society Hospital, doses were administered corresponding to those advocated by KIKUTHI as having been successful in the treatment of monkeys, namely 5 mg. per kg. twice with a 3-day interval. Thus, on the assumption that the average weight of adult males was between 70 and 80 kg. patients received two or three doses of 400 mg. with a 3-day interval between doses. In the later trials, which were carried out in part at the Endemic Diseases Hospital, in part among workmen of the Bilharzia Snail Destruction Section and in part among patients from the village of Saqeti, in their homes or during their temporary accommodation in the Oosun District Hospital, the drug was administered more frequently and in a heavier total dosage. Altogether six groups of patients received treatment with miracil D.

The first group consisted of 20 patients at the Endemic Diseases Hospital in fairly good general health and as far as possible free from intercurrent disease. Fourteen of these patients were suffering from haematobia infection alone, two from mansonii infection alone, and the remaining four from both forms of the disease. After the investigation of the liver and kidney function and the blood picture, two doses of 400 mg. separated by a 3-day interval were administered. Since most of the patients were under the assumed average weight, the actual rate of administration ranged from 7 to 10 mg. per kg. per dose. For the first 3 weeks after treatment the patients remained in the hospital under continuous observation. They were then discharged and thereafter reported for examination once a week, the total period of post treatment observation varying from 42 to 67 days. Since egg counts from untreated patients showed considerable daily fluctuation and since, where mansonii infections are concerned, the distribution of eggs in the faeces is apt to be very uneven, it is evident that examination once a week is inadequate and conclusions based thereon can only be regarded as tentative. However the results obtained give us reason to hope that in higher and more frequent dosage a large percentage of cures might be obtained. Apparent cures were effected in seven out of 20 cases which remained under supervision for the full period of the trials. In two cases haematobia ova disappeared from both urine and faeces by the 65th day after the end of treatment. In another case dead haematobia ova only were found

in the urine from the 56th day onwards. In the four other cases *mansoni* ova disappeared from the faeces or were all degenerate, but viable *haematobia* ova still occurred in the urine. Thus it would appear that out of six *mansoni* cases, four (67 per cent) were cured, while out of 18 *haematobia* cases only three (17 per cent) were cured. It seemed to us that this discrepancy might have been due rather to the situation of the worms in the body than to any inherent difference in their sensibility to the drug, since adults of *B. mansoni* situated in the mesenteric veins would be subjected to the full concentration of the drug as it was absorbed from the intestine, while adults of *B. haematobia* would only be exposed to it in, probably, lower concentration, after its passage through the liver. This may be correlated with the fact that the proportion of degenerate ova in the *haematobia* cases increased up to the end of the fifth week after treatment and then decreased again, which showed that the drug had a depressing effect on the reproductive activity of the worms, but did not reach them in sufficient concentration to cause death.

The second group consisted of twelve patients at the Church Missionary Society Hospital, who were likewise in fairly good health, and free from inter-current disease and all of whom were suffering from *haematobia* infection. They received three doses of 400 mg per kg separated by 3-day intervals. The patients remained under observation for periods varying from 5 to 70 days, but on leaving the hospital they disappeared and no further follow-up examinations were possible. Apparent cures were observed in only two of these cases (17 per cent). In one of these cases all the ova appearing in the urine were dead and degenerate for a period of 3 weeks up to the time when the patient left the hospital on the 55th day after the end of treatment. In the other case, in which ova had been originally present in both urine and faeces, there was a progressive increase in the percentage of degenerate ova from the first week after treatment, culminating in their complete disappearance from both urine and faeces on and after the 30th day. All other cases continued to pass viable ova up to the end of the period of observation.

The third group consisted of patients from the village of Saqeel, where a high incidence of *mansoni* infection had been reported. One of the difficulties in treating patients in their own homes was the likelihood of infection being re-acquired, which might mask the effect of so slowly acting a drug as miracidium. In this case, however, it was considered that the risk of reinfection developing in selected individuals was negligible since, although infected *Planorbis boissyi* had formerly been abundant in this area, all the canals surrounding the village had recently been cleared and treated with copper sulphate. Fifteen suspected cases were selected for examination, none of whom was found to be positive by sedimentation and six of these were sufficiently heavily infected to be detected by faecal smear alone. Four of the six heavily infected individuals were transferred as voluntary patients to the Oosim District Hospital, where they remained

as in patients for a fortnight. During the first week of this period each patient received four doses of 400 mg of miracil D administered on alternate days. After observation for a further period of a week they were allowed to return to their homes where they were visited, as far as possible weekly for clinical examination and the collection of faecal samples for laboratory tests. Ova both living and dead, were observed in the faeces until the seventh examination on the 50th day after treatment when only dead ova were found in three out of four patients. Clinical improvement was marked. A second course of treatment consisting of 400 mg administered on each of 5 successive days, was then given. Post treatment examination after this second course revealed only dead eggs on each occasion and hatching tests proved negative.

A fourth group consisting of six children from the village school of Saqee all of whom were haematobia cases, were later given treatment. The drug was administered 12 hourly in doses which varied from 100 to 300 mg according to the age and weight of the patient. The first course consisted of 5 or 6 days and, since the urine remained positive, a second course was given about 5 weeks later in which the dose was increased to from 200 to 300 mg in every case. The urine continued to be positive, although there was a decrease in the total number of ova and an increase in the proportion of degenerate ones. This result is of interest and significance since it would seem to indicate that in children, in whom the kidney function is generally good, the elimination of the drug is so rapid that it has little curative effect.

The fifth group consisted of workmen or the children of workmen attached to the Bilharzia Snail Destruction Section of the Ministry of Public Health who were known to be suffering from urinary bilharzia disease. The first case was a boy aged 14 years, weighing 43 kg. who had only a moderate infection but showed considerable haematuria. A 200 mg dose of miracil D was given every 12 hours up to a total of 2.4 grammes. Ova disappeared from the urine within 3 days of the end of treatment, while the haematuria disappeared even earlier. The physical condition of the patient improved and he no longer complained of pain on micturition. No subsequent relapse was observed. The second case was a man, 27 years old, weighing 75 kg. and suffering from a light and symptomless infestation. Haematuria was negligible. Six doses of 400 mg were administered over a period of 3 weeks, following which ova were absent from the urine for nearly 2 months, but later there was a relapse. A second treatment again produced temporary improvement, but a further relapse occurred later.

The third case was a boy aged 6½ years, weighing 15 kg. who was also very lightly infested and showed no haematuria. He received a 12 hourly dose of 50 mg over a period of a fortnight. Ova disappeared from the urine before

This man showed blood-level of miracil D of 10 µg per 100 ml. 48 hours after his last dose.

the end of treatment, but reappeared 2 months later. Ten further 12-hourly doses of 150 mg again produced disappearance of ova but there was a second relapse 2 months later. The fourth case was a youth 19 years old, weighing 58 kg, very heavily infected and showing severe haematuria. He received a 12-hourly dose of 300 mg for a period of 13 days, during which no less than 7.8 grammes was administered. The haematuria disappeared before the end of treatment and the ova were greatly reduced in number, the urine eventually becoming negative a week after the end of treatment. Three weeks later, however, there was a slight relapse, small numbers of viable ova reappeared in the urine but there was no further haematuria. Further treatment of nine 12-hourly doses of 600 mg reduced the ova to a negligible figure but did not produce a complete cure. This patient showed a striking improvement in physical condition. Similar results were obtained in five further cases. Two men suffering from the intestinal form of the disease received sixteen to eighteen 12-hourly doses of 5 mg per kg, although the number of ova and the amount of blood in the faeces was reduced a complete cure was not obtained. Three haematobium cases, all children, received treatment, which was repeated after an interval in two of these. Although haematuria disappeared, there was a reduction of ova in one case only and no complete cure in any of them.

After a consideration of the results of this group of cases three points arise. In the first place, improvement was substantially less marked in the children, a fact which may be correlated with the efficient kidney function and rapid elimination of the drug. In the second place, since the degree of improvement was less marked in lightly infested cases it may be that the drug is possibly rapidly metabolized by the undamaged liver, whereas in heavily infested cases the degree of liver damage is possibly such that the drug can only be metabolized more slowly and therefore continues to circulate in adequate concentration in the blood for a longer period. In the third place, the fact that partial relapse occurs gives rise to the supposition that while some of the worms are killed by the drug, others merely undergo temporary interruption of reproductive activities and subsequently recover.

The sixth and last group consisted of a further 14 patients in the Endemic Diseases Hospital who received treatment at the rate of 5 mg per kg every 12 hours if the urea clearance rate was above 50, and 2.5 mg per kg if it was below this figure. Treatment was continued for from 5 to 7 days, so that some patients received a total dose of as much as 70 mg per kg. The patients remained in hospital for a fortnight after the end of treatment before their discharge and, up to this time, all were still positive although both the number of ova and the degree of haematuria had greatly diminished. An attempt to grade the rate of dosage in accordance with the urea clearance rate was made in view of the fact that it had been shown by HALAWANI, NIWSOMR and WOOTTON (1947) that there was a significant positive correlation between this figure and

the apparent level of miracil D in the blood. It must be pointed out, however, that the tests for estimating the blood level of the drug are not specific and it may well be that the figure which they reveal includes metabolic products formed in the liver.

Further trials are in process of being carried out at the Endemic Diseases Hospital and elsewhere.

DISCUSSION

The experimental and clinical results recorded above appear to permit the conclusion that miracil D administered by the oral route is effective against both *B. haematobia* (urinary bilharziasis) and *B. mansoni* (intestinal bilharziasis and Egyptian splenomegaly) provided that a sufficient total dosage is administered over a sufficiently short period of time, but although marked improvement is apparent complete cure is not usually attained.

However, the drug appears to be erratic in its action, curing some experimental animals and some patients and failing to cure others. These erratic results produced by miracil D in the treatment of both experimentally infected animals and human patients when low dosages were used suggested that the blood level of the drug might vary in different individuals after the same dose. HALAWANI, NEWBOUR and WOOLTON (1947) investigated this point in human patients. They found that after a single dose of 400 mg. miracil D level in the blood rose to a peak value which varied from below 50 to over 500 μg . per 100 c.c. between 12 and 24 hours after administration, depending on the efficiency of the kidney function. In cases with a high peak the blood level dropped slowly and could still be above 100 μg . per 100 c.c. 48 hours after administration, while in cases with a low peak it dropped so rapidly that it could reach zero within 24 hours. In some of our cases which had received repeated administration it was above 200 μg . 48 hours after the last dose. Clearly the suggestion made very early in the investigation by one of us (M.A.V.) that dosage every 12 hours would probably prove to be most satisfactory was borne out by the results of the blood level tests.

Further work showed that a statistical correlation exists between blood miracil D level and urea clearance rate. Thus it would seem a wise plan to adapt the dosage to the urea clearance rate in order to maintain a high level of miracil D in the blood over a period of a week or more so that the parasites might be subjected to a lethal concentration of the drug for a sufficient length of time.

Several parallels exist between effects observed in connection with antimony treatment to those observed in connection with miracil D. Thus as with miracil D so in treatment with fusidin and tartar emetic the blood level of the drug varies according to the excretion rate in different individuals. Further it is a fact that the relapse rate is higher in children after treatment with antimony

compounds, probably because the kidney function is better and the drug is excreted more rapidly. The results of BLAIR, HAWKING and ROSS (1947) in obtaining a small percentage of cures in children suggest that this may well prove to be true with miracid D also.

The possibility should not be overlooked that immature worms, due perhaps to their physical position in the body of the patient, are apparently unaffected by the drug and still go on to maturity and produce eggs in due course. Thus the fact that the original mature adults had been killed would be masked when the immature worms were present at the time of treatment, or reinfection took place during or immediately following treatment. This factor may be possible to avoid by interrupting treatment and continuing it after an interval, as in the treatment of oxyuriasis with gentian violet.

From the clinical point of view the rapid disappearance of haematuria in patients receiving miracid D treatment is important since it indicates that whether or not a complete cure is obtained, the patient shows marked improvement in physical condition. In this connection it is of interest to note that tartar emetic treatment is often preferred by patients to treatment with furidin on account of the fact that the disappearance of haematuria is much more rapid with the former drug.

It may be not without interest to compare our results with those of BLAIR, HAWKING and ROSS (1947), who reported that 2 months after the end of treatment 86 per cent. of their patients who had received miracid D were still passing living ova, and therefore concluded that the therapeutic action of the drug was slight. However, they stated that the maximum tolerated dose for repeated administration was 200 to 300 mg per day whereas in our experience as much as 600 mg per day can be administered over a period of a fortnight without ill effects. It is true that some patients had idiosyncrasy, but in other cases much higher dosage than that used by HAWKING and his collaborators can be safely employed. Moreover, it is doubtful whether a satisfactory conclusion can be based on examination at fortnightly intervals. These workers probably failed to administer a sufficient dose rate to maintain the minimum necessary blood level of the drug to kill the worms. Most of the patients were school children, and presumably had good kidney function. On the basis of the tests carried out by HALAWANI, NEWSOM and WOOTTON (1947) at the Fouad I Research Institute in patients of varying ages, it may be concluded that with such dosage used in children a blood level of about 25 to 50 μg per 100 c.c. would be all that could be expected. It is practically certain that such a blood level is far below the threshold value to kill the worms.

We, also, observed that improvement was substantially less marked in children than in adults receiving the same dosage rate per kg.

In our clinical trials, better results were obtained in the treatment of mansonii infestation than in haematobia cases. BLAIR, HAWKING and ROSS

found no significant difference in the cure rate of the two varieties of the disease. It may be, as pointed out earlier, that the drug reaches worms in the mesenteric veins in higher concentration than those in the veins of the venal plexus.

In cases in which miracil D fails to effect a cure of bilharzia disease it does not necessarily mean that the drug has been ineffective. It may be that the dose was insufficient or that administration should have been continued for a longer period.

Whether or not further clinical trials bear out our preliminary tentative conclusion that miracil D administered orally is effective in the treatment of *B. haematobia* and *B. mansoni* infections when used in a sufficient dosage, it is at least possible that it will prove to be the starting point of a new field of research in this sphere which may ultimately culminate in the production of an oral drug which will kill the parasites in man quickly, surely and safely.

SUMMARY

1. The history of attempts to develop a drug for the oral treatment of bilharzia disease in human beings is briefly reviewed, concluding with an account of the development of the new antibilharzial drug miracil D (khodin).

2. A description is given of tests in which animals experimentally infected with *B. mansoni* and *B. haematobia* were treated with solutions of miracil D administered by the oral route. In those animals to which a sufficient dosage was given over a sufficient length of time, for example, five doses of 40 to 50 mg per kg, both viable ova and the symptoms of the disease disappeared and at autopsy only dead and disintegrating worms were found. However in lower dosages the drug appeared to be erratic in its action, killing some of the worms and curing some animals when used in a given dosage while failing to kill others.

3. An account is also given of clinical trials in which miracil D was administered to human patients suffering from both varieties of bilharzia disease. Here, also, in lower dosages the drug appeared to be erratic in its action, curing some patients and failing to cure others, but the results of the later trials, in which higher and more frequent doses were given, were more consistent and improvement was more marked. In the earlier series of trials, doses of 400 mg were administered twice or thrice at 3-day intervals. In the later series of trials, doses of up to 300 mg at 12 hourly intervals were given for as long as a fortnight. Viable ova disappeared from the urine or faeces, haematuria vanished and the physical condition of the patient substantially improved, but most cases later showed partial relapse. It is impossible to generalize too widely with regard to dosage since this varied according to the weight, physical condition and urea clearance rate of the patient. Further improvement was achieved after second treatment, and although few complete cures were obtained the reduction in the number of ova and the amount of blood in urine or faeces was considerable. The most satisfactory clinical results were achieved by keeping

the blood miracid D, as we believe, up to the lethal level for the parasites by administration every 12 hours. The minimum effective dosage seemed to be 5 mg per kg every 12 hours for a minimum period of 5 days although a higher rate is almost certainly desirable.

4 The pharmacology of miracid D is briefly reviewed and the toxic symptoms observed during administration to human patients are recorded. Such toxic symptoms are generally slight and appear only to affect one out of five or six patients. These toxic symptoms include anorexia or even vomiting, abdominal pain, and, sometimes, giddiness and noises in the ear. Whether or not these toxic symptoms are associated with some undetermined contra-indication or whether they are due to insufficient kidney function leading to undue accumulation of the drug in the body and an exceptionally high blood level has not yet been discovered.

5 Contra-indications have yet to be determined but no obvious ones have been observed, although it is probable that administration of miracid D would prove to be incompatible with heart disease or organic disease of the kidneys.

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TRANSACTIONS OF THE ROYAL SOCIETY OF
TROPICAL MEDICINE AND HYGIENE
Vol 42 No 1 July, 1948

A CLINICAL AND SEROLOGICAL FOLLOW-UP OF YAWS CASES TREATED BY ACETYLARSAN AND BISMUTH SODIUM POTASSIUM TARTRATE

BY

I APTED

AND

R D HARDING,

Yaws and Sleeping Sickness Campaign

AND

M GOSDEN,

Senior Pathologist, Sierra Leone

This paper deals with cases of yaws which had been treated at various times up to $2\frac{1}{2}$ years previously. The clinical observations and Ide tests were carried out by two of us (I A and R D II) in the field, while the Kahn tests were made by the third member (M G) in the Government Laboratory, Freetown. The object of the investigation was to obtain an indication of the cure rates following the use of acetylarsan and bismuth sodium potassium tartrate (B S P T) alone and in combination. The study was undertaken in two areas separated by about 70 miles. In the first area the results after both drugs, alone and in combination, were examined at approximately the same time after treatment, in the second, a comparison was made of the results obtained at varying intervals after treatment by the standard combination of acetylarsan and B S P T in use in the yaws campaign. At the time of the clinical examination Ide tests were carried out on the spot, and as many sera as possible were sent to Freetown for Kahn tests.

Serological results of treatment by B S P T have been recorded by CARMAN (1928), who found in a small series of cases that after 12 injections of this drug a large percentage were left with positive serum reactions. FITZGERALD and GUPTA (1934) tested a number of bismuth preparations but found them

* Our thanks are due to Dr B G T ELMES, FRCP, of the Medical Research Institute, Yaba, Nigeria, for supplying us with the Ide antigen, and to Dr W P H LIGHTBODY, CBE, Director of Medical Services, Sierra Leone, for permission to publish this paper. Acknowledgement is also due to Drs MICHIE, HUTCHINSON and PEASTON, who (when working in the yaws campaign) treated and recorded the initial lesions of some of the patients.

all of little value in effecting persistently good results however when they combined 2 or 3 injections of neosalvarsan with 8 injections of bismuth, serological cure was obtained in half the cases. WILSON (1937) records results of Kahn tests after acetylarsan, but it is not clear from his paper at what periods after treatment the tests were carried out. Apparently of thirty-one patients, all Kahn positive before treatment, who received between 4 and 11 doses of the drug, seven had become Kahn negative on the occasion of the last subsequent test carried out but the interval between treatment and the last test may in some cases have been too short for reversal to be expected. Clinically most of his cases were cured and the remainder improved. In our series serological tests were not carried out before treatment, but clinically all the cases were considered to be definitely yaws, and the fact that only 7 per cent. of cases were Kahn negative after ineffectual treatment with B.S.P.T. makes it reasonably certain that practically all our cases would have been positive had they been tested before treatment.

COMPARISON BETWEEN RESULTS OF KAHN AND IDE TESTS.

In carrying out the Ide test the method described by SMITH and ELMS (1945) was first followed, using unheated serum, but it was found that many of the positive Kahn sera were Ide negative. After deactivating the sera at 56° C. results were somewhat better but there was still a considerable measure of disagreement. The original Ide (1936) technique quoted by GRADWOLD (1938) was then tried. Correspondence was closer but the most successful batch still showed an agreement of only 80 per cent. Finally we came to the conclusion that deactivation of the serum was essential and that it was also advisable to add a drop of 3.5 per cent. saline to the serum on the slide.

The method used was to add platinum loopful of the serum to be tested to a loopful of 3.5 per cent. saline in the concavity of slide, and then drop of the diluted Ide antigen from a pipette (the substitute platinum loops available took up too small a quantity of the antigen, whose surface tension is less than that of the other reagents). The reagents were mixed with the corner of cover slip after which the slides were shaken with t and fro and circular movements for 5 minutes and the results then read immediately with the naked eye. An example of the comparative Kahn and Ide results is shown in Table I.

TABLE I
COMPARATIVE RESULTS KAHN AND IDE TESTS.

Sera tested.	Kahn result	Corresponding Ide result
41	1 to 4 +	31 positive 10 negative
8	Doubtful	8
7	Negative	2
Totals	41 pos. 8 doubtful, 7 neg	39 positive, 17 negative

Considering only those sera whose Kahn reactions were either definitely positive or definitely negative, it will be seen that there was a 75 per cent agreement between the two tests. When, however, we tested a number of sera from untreated cases of active yaws or from cases which had failed to respond clinically to treatment on the one hand, and from a few subjects known to be free from syphilis or yaws on the other, practically 100 per cent agreement was obtained. In our hands (I A and R D H) the Ide test was not very satisfactory as a method of estimating serological cures some months after treatment when the titres of many of the sera had become reduced, and it appears that the Ide reaction would have a better field of application in testing an untreated community for yaws or syphilis when most of the sera would presumably be either strongly positive or completely negative. (Possibly better results would have been obtained if we had measured the drops of serum, saline, and diluted antigen accurately from a pipette.) The necessity for deactivation of the serum also considerably reduced the simplicity of the test when practised in the field. Another difficulty encountered was that on hot dry afternoons the test reagents on the slide tended to dry up during shaking before the results could be read. We feel some doubt as to the reliability of the test when practised by African technical assistants as SMITH and ELMES (1945) suggest, at least if it is to be used as a test for cure after treatment and not merely as a diagnostic confirmation.

CLINICAL AND SEROLOGICAL RESULTS OF FOLLOW-UP AFTER ACETYLARSAN AND B S P T ALONE AND IN COMBINATION

The cases shown in Tables II and III were examined 6 to 7 months after the commencement of their treatment. Injections had been given at weekly intervals. Nearly all the patients received the full course shown, a few missed one injection. The sera for Kahn tests were collected at the time of the clinical examination, the number of tests done is less than the number of people examined as some of the sera became spoilt during transmission. In Table II active non-infectious plantar yaws signifies marked lesions such as hyperkeratosis, erosions, fissures, etc., accompanied by pain on walking, inactive plantar yaws signifies similar lesions, but usually much less marked and unaccompanied by symptoms. Bony changes were not taken into account. In actual fact, nearly all patients who at follow-up showed any lesions other than framboesiomata showed plantar changes in addition and so are included in the plantar category.

The clinical and serological results after the different courses are seen to run roughly parallel, acetylarsan alone in six doses producing most successes, both clinically and serologically, and B S P T alone the fewest. In Sierra Leone much the commonest form of yaws encountered is non-infectious plantar yaws comprising hyperkeratosis, clavus, cracks and fissures, erosions, etc., and of the present series of 274 patients followed up, 235 had at the time of treatment

TABLE II.

FOLLOW UP TO 6 MONTHS AFTER TREATMENT

Drug	Dosage	Cases	Per cent. in lesions as under				
			Infectious	Non-infectious planter	Total active	1 active planter	No lesions
Acetylarson	6 4 c.c.	85	6.0	0.0	0.0	29	81
Acetylarson plus	3 3 c.c. plus						
B.S.P.T	4 3 grams	120	3	0.9	3.3	4	1
B.S.P.T	6 4 grams	36	8.9	23.1	24.0	21	22

TABLE III.

FOLLOW UP TO 6 TO 7 MONTHS AFTER TREATMENT

Drug	Dosage	Cases	Per cent. in results under				
			3 to 4	1 to 2	Weak	± or doubtful	No signs
Acetylarson	6 1 c.c.	7	1	0	13	12	1
			80				
Acetylarson plus	3 3 c.c. plus						
B.S.P.T	4 3 grams	9	30	0	4	11	7
			7				
B.S.P.T	6 4 grams	15	71	16		4	7
			87				

been suffering from late secondary yaws of this type, while only 22 exhibited frambœs omata. The remaining 17 had infectious planter yaw of pseudo-granulomatous type (crab yaws). The results of treatment by any one course in these three types of lesion were analysed separately but as no significant difference was found they have been combined in the above tables. No cases of tertiary yaws were included. Most of the lesions encountered at the follow up were of non-infectious planter type and in some cases it was difficult to decide whether slight irregularities of the soles were due

to yaws or to trauma induced by climbing palm trees, swamp farming and the like, but where doubt existed the case was classified as inactive plantar yaws. It was interesting to find that many patients who had shown gross hyperkeratosis and erosions before treatment exhibited at the time of the follow-up perfectly supple normal soles, particularly after acetylarsan. We had previously been in doubt whether such gross lesions did not represent irreversible epithelial damage which might be expected to persist even after eradication of the spirochaetes. However, in a few cases minor plantar lesions persisted though the Kahn reaction had become negative.

The results indicate that acetylarsan is a moderately effective drug, though the proportion of negative Kahns (if doubtful reactions are excluded) is not as high as that of negative Wassermanns obtained by the JAMAICA YAWS COMMISSION (1934-36) 6 months after a course of four to five injections of either neoarsphenamine or bismuth salicylate in oil. It is evident that B S P T does little more in the doses given than procure temporary amelioration in a proportion of cases since no less than 71 per cent of cases treated with this drug alone still gave a three plus or four plus Kahn reaction. There is also no evidence that B S P T exerts a synergic effect when combined with acetylarsan since the results are no better after the combination than might have been expected with 3 x 3 c c of acetylarsan alone, and are in fact inferior to those obtained after 6 x 4 c c acetylarsan. HILL *et al* (1946) remark in connection with the high rate of persisting positive Kahn reactions after treatment of their cases with penicillin, that there appears to be considerable uncertainty as to how far serological reactions in yaws cases are a significant guide to ultimate cure. Yet in five primary cases which were Kahn positive before treatment they obtained reversal in all, and in a sixth originally Kahn negative the reaction remained negative. Again, the fact that the JAMAICA YAWS COMMISSION obtained Wassermann reversals in more than half of their infectious, and in nearly half of their non-infectious cases 12 months or more after treatment would seem to resolve this uncertainty. Judging purely on the basis of the serological results recorded in the reports by HILL *et al*, the JAMAICA YAWS COMMISSION, and the present paper, it would seem that, at any rate in secondary yaws, penicillin in the doses stated is inferior to neoarsphenamine, bismuth salicylate, and acetylarsan. It is to be remarked that there is little likelihood of ever evaluating a sound cure rate on clinical grounds alone in primitive communities, since a single injection of a number of drugs will often clear up lesions for the time being and, even when untreated, yaws may undergo long periods of latency, sometimes extending into years, between successive eruptions. The matter is further complicated by the marked seasonal variations in yaws manifestations which occur. Probably it would be necessary to re-examine cases three or four times a year for several years before cure could be established on clinical grounds alone.

In consequence of the poor results obtained after B S P I, this drug has

now been discarded in the yaws campaign in Sierra Leone and bismuth salicylate in oil has been substituted. The contrast in the immediate clinical results is striking, these being about as good as after acetylarsan. Sufficient time has not yet elapsed to evaluate the serological cure rate.

It will be noted from Table III that a high proportion (30 per cent.) of the cases which had been treated with acetylarsan only fell into the Kahn group \pm + doubtful, or \pm weak. This led to the suspicion that many of the cases were in course of reversal at 6 months, and given more time might become completely negative. Accordingly thirty seven cases were re-tested a further 6 months later i.e. about 1 year after treatment and the results are shown in Table IV. Most of these cases had received acetylarsan only a few the combined course

TABLE IV

CASES OF YAWS KAHN RESULTS 6 MONTHS WHEN REPEATED 1 YEAR IN SAME INDIVIDUALS

	Kahn at 6 months.				Corresponding Kahn at 1 year			
	3 + 4 -	1 + to 2	\pm Weak or doubtful	Neg- ative	3 + 4	1 + 2	\pm Weak or doubtful	Neg- ative
Cases	9	12	11	3	8	3	—	—
					4	6	—	2
					3	6	2	1
					1	—	—	2
Total	9	12	11	3	14	16	2	3

The results show no tendency towards an increasing proportion of Kahn reversal with time. In fact, most of the doubtful Kahns at 6 months became definitely positive at 12. The Kahn results after still longer periods in other sets of cases are dealt with below.

CLINICAL AND SEROLOGICAL RESULTS AT FROM 6 MONTHS TO 2½ YEARS AFTER TREATMENT WITH STANDARD COMBINATION.

This follow up was carried out in the other area. In the area dealt with in Part I only the worst cases were taken for treatment in a community in which treatment facilities had been available for some years previous and many of the cases represented plantar relapses after one or more injections received at some time in the past. In the area now under consideration previous treatment had not been so readily available and as a consequence there was a higher proportion of infectious yaws. In this area all cases considered

have definite signs of yaws were treated whether or not they complained of symptoms, but cases of tertiary yaws are omitted in the following analyses. Clinical re-examinations were carried out in 1945 and 1946 on different batches of patients treated in 1943, 1944, and 1945. All the Kahn tests were carried out in 1946 on as many cases as was practicable from the same batches. The treatment given comprised the standard course in use at the time. Up to the end of 1943 the adult dose of acetylarsan was 5 c.c. at each injection, but at that time a number of toxic reactions occurred and it was found necessary to reduce it first to 4 c.c. and later to less than 3 c.c. to avoid them. The dose of B.S.P.T. was 3 to 4 grains. Children received proportionately lower doses according to body weight. The acetylarsan and B.S.P.T. were given concurrently, one into each buttock, at 5-day intervals. Results of the follow-up are shown in Tables V and VI. In the classification adopted for recording clinical results active yaws includes framboesiomata, pseudo-granulomatous plantar yaws, and non-infectious plantar yaws accompanied by pain, the term inactive plantar lesions refers to marked non-infectious plantar yaws unaccompanied by pain, slight plantar irregularities comprised minor lesions probably in most cases attributable to nearly healed yaws, but in a few cases possibly due to occupational trauma.

TABLE V
CLINICAL RESULTS AT DIFFERENT PERIODS AFTER TREATMENT

				Per cent with lesions as under			
Period since treatment	Course	Initial lesion	Cases	Active yaws	In-active plantar lesions	Slight plantar irregularities	No lesions
6 months	A3 \times 2 c c plus B4 \times 4 grains	Infectious	27	0	7	22	70
		Non-infectious	33	3	6	33	58
1 year	A4 \times 4 c c plus B4 \times 3 grains	Infectious	75	1	3	32	64
		Non-infectious	108	5	10	51	34
2 years	A4 \times 5 c c plus B4 \times 4 grains	Infectious	90	1	2	21	76
		Non-infectious	154	2	11	43	45
All periods	—	Infectious	192	1	3	26	70
		Non-infectious	295	3	10	44	43
All periods infectious and non-infectious			487	2.3	7.4	36.8	53.6

TABLE VI.
KAHN REACTIONS DIFFERENT PERIODS AFTER THE TREATMENT

Period since treatment	Course	Initial lesion	Per cent. with Kahn result at					Neg.
			Cases	+	-	at	or	
				3	1	4	1	
6 months	A4 2 c. plus B4 4 grains	Infectious	27	24	3	1	11	22
		Non-infectious	25	9	16	6	55	50
1½ years	A4 4 c. plus B4 3 gr. wry	Infectious	29	2	27	1	1	31
		Non-infectious	4	3	1	1	15	45
1½ years	A4 5 c.c. plus B4 4 grains	Infectious	23	6	17	2	14	17
		Non-infectious	21	6	15	20	—	—
All period	—	Infectious	91	11	80	18	14	31
		Non-infectious	146	6	140	12	1	25
All period	infectious and non-infectious		237	17	220	30	15	46

Tables V and VI show no significant difference in either the clinical or the serological results whatever period had elapsed since treatment. This is the more surprising since the cases re-examined after 6 months had received a much smaller dose of acetylarsan than those in the other two groups. It will be observed from Table V that cases treated in an infectious stage consistently showed a smaller proportion of lesions of any sort at follow up than those treated in a non-infectious stage. Frequent observations of this sort in the course of the yaws campaign had led us to believe that yaws is more readily cured in the early frambœus stage than in the generally later stage when marked epithelial changes of the soles have developed but the Kahn results do not support this belief. In fact the Kahn reaction was negative at follow up in 31 per cent. of cases of the former type at all periods and in 25 per cent. of those of the latter.

The higher proportion of negative Kahn reactions in this area than among the acetylarsan-treated cases in the first area may possibly be due to the fact that in the first area only the worst cases were taken for treatment whereas in the second all cases diagnosed as yaws were taken.

CONCLUSIONS

As previously remarked, the only satisfactory method of estimating the clinical relapse rate after treatment is to carry out frequently repeated re-examinations of the patients over a long period, since otherwise relapses which occur but clear up spontaneously may be missed. In our series the patients treated in 1943 were re-examined in 1944 as well as in 1945, but those treated in 1944 and 1945 were only seen again at the final examination. However, all patients were asked whether they had had any relapses and whether they had sought subsequent treatment at any hospital or dispensary, and from the results of these inquiries it appeared that relapses other than those recorded were infrequent.

It seems fair to conclude that acetylarsan is very successful in banishing symptoms and the more marked signs of yaws for at least $2\frac{1}{2}$ years, and the bulk of those signs which remain comprise in this country minor inactive plantar lesions. On clinical grounds alone it is impossible to express an opinion as to the probable ultimate cure rate. If the serological findings are combined with the clinical, the picture which presents itself is one in which perhaps one-third of the cases are permanently cured by acetylarsan in the doses given, and the majority of the remaining two-thirds are rendered quiescent for an indefinite though prolonged period.

It is interesting to compare the serological results obtained by the JAMAICA YAWS COMMISSION (1936) in yaws cases treated with an average of four to five injections of neoarsphenamine or bismuth salicylate in oil. This Commission found that an increasing number of their cases became Wassermann negative with lapse of time after treatment. The figures are complicated by the fact that some cases who relapsed received additional treatment before their final tests, but it was estimated that if no additional treatment had been given the probable proportion of Wassermann negatives after $2\frac{1}{2}$ years would have been 49 per cent following neoarsphenamine, and 60 per cent following bismuth salicylate. The response to treatment may differ somewhat in Sierra Leone, and in our series the high proportion of doubtful or weak Kahn reactions makes interpretation difficult, but the comparison suggests that acetylarsan is inferior to neoarsphenamine and still more so to bismuth salicylate. It is hoped to investigate serologically a batch of cases recently treated in Sierra Leone with bismuth salicylate to provide a comparison between the results with this drug and with acetylarsan in similar types of cases in the same region.

One point deserves further reference—the great contrast between the therapeutic effects of bismuth salicylate and B S P 7. It was expected that these two drugs when given in equivalent dosage would produce similar results, but in practice the difference as regards the immediate clearance of lesions has been striking.

SUMMARY

The results are described of a clinical and serological follow-up of yaws cases treated from 6 to 30 months previously by acetylsan and bismuth sodium potassium tartrate (B.S.P.T.) alone and in combination. B.S.P.T. was shown to be relatively ineffective. Acetylsan was very effective in clearing lesions and in preventing active relapses for 2½ years, though over one-third of the cases continued to show minor plantar irregularities probably attributable to yaws. In one area the proportion of cases with negative Kahn reactions 6 months after treatment with courses containing acetylsan was 17 per cent., though 43 per cent. gave reactions of less than one plus. In another area in which cases were tested from 6 to 30 months after treatment, the corresponding figures were 32 per cent. and 68 per cent. It is concluded that acetylsan in the doses given probably cured in the neighbourhood of one third of the cases and rendered the majority of the remainder quiescent for an indefinite though prolonged period.

The correspondence between the Ide and the Kahn tests was not sufficiently close in previously treated cases to justify reliance on the former

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ANAEMIA AND MARASMUS IN INDIAN TROOPS ON ACTIVE SERVICE

BY

R. H. GIRDWOOD, MCHB, FRCP, MRCS.
(formerly Lieut. Colonel R.A.M.C.)
Lecturer in Medical Geography (Edinburgh)

During the recent campaign against the Japanese, one of the problems with which the medical services were faced was that of anaemia amongst Indian troops. An account of the extent of iron-deficiency anaemia amongst Indian troops in North-West India has been given by HAYES and ISHAG (1945), who found that two-thirds of 1356 North-West Indian soldiers had less than 14 grammes haemoglobin per 100 ml. JAYSON and CUNNINGHAM (1945) have described the picture of nutritional macrocytic anaemia in fifty Indian males, all of whom were found to be vegetarians.

The present report is based on the findings of a preliminary investigation into anaemia in Indian troops in various military hospitals in Bengal commenced in the early part of 1945. The investigation was carried out at the instigation of G.H.Q. (India) because of the prevalence of a syndrome of marasmus and anaemia, with associated evidences of vitamin deficiency almost entirely confined to Indian troops. This condition has been referred to by MAURIOTT (1945). The object of the investigation was to define the clinical features, and to describe the type of anaemia in modern haematological terms. The scope of the investigation was limited: cases were seen in various transit hospitals interspersed between the fighting area of Burma and the base hospitals in India. Accordingly, some of the patients were not seen until they were on the road to recovery, since they had been too ill to evacuate from the forward hospitals during the more acute stage of the illness. No deaths occurred amongst the present small series, but a fair result was not uncommon in forward hospitals, and also in base hospitals where men were admitted either at the end of the chain of evacuation or when on leave from the front.

* I am grateful to the DIRECTOR GENERAL ARMY MEDICAL SERVICES, for permission to publish this paper, to Brigadier (now Major General) I. HARRIS and Dr. A. M. THOMSON, (formerly Lieut.-Colonel R.A.M.C.) for the interest they took in the work, also to Dr. I. G. W. HILL (formerly Brigadier Consultant Physician, Fifth Army and A.L.F.S.E.A.), and Professor L. S. P. DAVIDSON for their helpful criticism and advice.

The syndrome of wasting and anaemia had a seasonal incidence, cases being more numerous towards the end of the monsoon. It was largely confined to troops stationed east of the Brahmaputra river. The aetiology was obscure. The present investigation was commenced several months after the 1944 monsoon when the problem had been very acute and the number of cases passing through the transit hospitals was very much less than it had been in the preceding months.

THE EXTENT OF ANAEMIA AMONGST INDIAN TROOPS IN HOSPITAL

In May 1945 a haemoglobin survey was carried out on 500 Indian troops of various races and castes, passing through a transit hospital in Bengal. The cases were unselected, except that no acute surgical cases and no patients suffering from acute malaria were included. Estimations were carried out on a Sahli instrument that had been standardized for a 5-minute reading. Capillary blood was used. The results are summarized in Table I.

TABLE I.

THE DISTRIBUTION OF Hb ALUES (CAPILLARY BLOOD) IN HOSPITAL POPULATION OF INDIAN TROOPS.

	Haemoglobin (grammes per 100 ml.)																Total	Mean
	3-	4-	5-	6-	7-	8-	9-	10-	11-	12-	13-	14-	15-	16-	17-			
No. of cases	1		1	3	7	11	12	40	21	31	6	105	180	36	21	400	14.17	
Per cent	4					3.2	2.4	4	4.2	6.8	1.5	21	20	11.2	4.2			

There was no significant difference between the figures for rice eaters,atta eaters and those who ate both rice and atta. The mean haemoglobin for 40 meat eaters was 14.19 grammes per 100 ml. and for 60 non-meat eaters it was 14.02 grammes per 100 ml. This difference is not statistically significant. It has frequently been stated that anaemia is more common amongst vegetarians in India. It should be noted that owing to supply difficulties and religious prejudices about the methods of preparation of meat, many of the non-vegetarian troops fighting in the jungles received only negligible amounts of flesh foods.

The above sample included both fighting troops and men from ancillary units, all of whom were being evacuated westwards to base hospitals in India. There was no significant difference in the mean haemoglobin values for various castes or units, except in the case of the Pioneers who were significantly lower than either the infantry or the other ancillary units. During the period of the investigation, however, the Pioneers passing through the hospital included an unduly high proportion of very recent recruits with less than a year's service, from a unit which had recently moved into the area drained by the transit

The 500 patients whose haemoglobin was estimated included 107 Pioneers with a mean haemoglobin of 13.25 grammes per 100 ml. The mean haemoglobin of troops with less than a year's service was low, as is seen in Table II, but since all but four of the cases with less than a year's service were from the Pioneer unit referred to above, it would be unwise to draw any conclusions about the effect of a year's service on the haemoglobin level. The differences between the other groups are not significant. It was not possible to examine the stools of all these men for the presence of hookworm.

TABLE II
SHOWING THE RELATIONSHIP BETWEEN MEAN HB AND LENGTH OF SERVICE

Length of service	Mean Hb (grammes per 100 ml)	Number of cases *	σ
Less than 1 year	11.89		
1-2 years	14.74	54	2.805
2-3 "	14.34	47	1.615
3-4 "	14.43	99	2.258
4-5 "	14.48	148	2.070
More than 5 years	14.83	82	2.410
		63	1.645

* The total number of cases is less than 500 because 7 of the men were civilians attached to the Army.

In June, 1945, a special anaemia centre was opened at a military hospital in Eastern Bengal for the diagnosis and treatment of anaemia, avitaminosis and malnutrition. All cases admitted to the centre during a period of 28 days in July, 1945, were seen, a haemoglobin estimation and a red cell count were carried out. Ninety-three new cases were admitted to the centre during this period from areas east of the Brahmaputra river. The blood figures were as shown in Table III.

It will be seen that many of these cases had a macrocytic form of anaemia. Sixty-one of the patients had stool examinations carried out for ancylostomes, by the concentration method, but in some this was done only once. Twenty-five cases, or 41 per cent, were positive, but it is likely that more would have been positive had it been possible to carry out repeated examinations. The mean corpuscular haemoglobin for 25 cases shown to be suffering from ancylostomiasis was 26.5 $\gamma\gamma$, and for the 36 whose stool examinations were negative it was 34.81 $\gamma\gamma$. This difference is significant. The mean haemoglobin for 27 non-meat eaters was 11.44 grammes per 100 ml and for 66 meat eaters it was 11.26 grammes per 100 ml. This difference is not significant. The numbers in the series are too small for analysis as regards various castes, etc.

TABLE III.

HAEMOGLOBIN AND RED CELL COUNTS OF INDIAN TROOPS ADMITTED WITH A DIAGNOSIS OF ANAEMIA, ASTHENA, OR MALNUTRITION.

Haemoglobin group (grammes per 100 ml.).	Mean Hb. (grammes per 100 ml.).	Number of cases.	Mean red cell count (per c.mm.).	Mean corpuscular haemoglobin (number of cases).		
				Less than 27	27-32	More than 32
2-6	—	—	—	—	—	—
3-4	6.6	1	2,000,000	1	—	—
4-5	4.6	—	1,993,000	1	—	1
5-6	5.7	2	2,340,000	2	—	—
6-7	6.6	4	2,253,000	—	1	1
7-8	7.4	3	6,092,000	4	1	1
8-9	8.8	6	3,053,800	6	1	2
9-10	8.6	10	2,234,000	6	6	4
10-11	10.4	16	3,740,000	5	3	5
11-12	11.8	16	3,435,000	—	5	6
12-13	12.6	7	3,817,000	—	1	6
13-14	13.6	7	4,274,000	—	2	4
14-15	14.6	11	4,837,000	1	4	6
15-16	16.4	9	4,932,000	—	5	4
16-17	16.6	6	4,230,800	—	—	3
Number of cases		93		—	29	43

THE MARASMUS SYNDROME.

One hundred and twenty four Indian other ranks, believed to be suffering from this syndrome were examined in the course of this investigation. The haematological findings of only 27 of these are recorded here, as satisfactory standardized apparatus was available only in the later stages of the investigation. The majority of the patients whose haematological figures are reported were included in Tables I or III. All the figures for this part of the haematological survey were estimated on venous blood which was drawn, without stasis, into Wintrobe's dry oxalate mixture.

It was difficult to define which cases should be included in the clinical survey as there were various gradations of the clinical features, and some patients had improved in the forward hospitals after prolonged treatment, prior to being seen in connection with the present investigation. All those chosen appeared to be suffering from a similar disease the chief presenting features of which were persistent diarrhoea, for which no cause could be found, wasting, glossitis, abdominal discomfort and anaemia, which was usually macrocytic in type.

Cases of dysentery, and straightforward cases of anaemia due to ancylostomiasis or to acute malaria, were not included in this part of the investigation

Duration of Symptoms—The exact time of onset of the first symptoms was difficult to elicit, but the answers given varied from 1 to 57 weeks, with an average of approximately 17 weeks, before the patients were seen in connection with this investigation

Place of Service—All but one of these patients had served in areas east of the Brahmaputra river, chiefly in Assam and Burma, and had never had such symptoms prior to going to those areas

Food Supply—MARRIOTT (1946) has referred to the importance of poor diet as a factor in the production of anaemia amongst Indian troops. Many of the men had had poor feeding for many years prior to joining the Army, and supply difficulties were great in jungle warfare. The only way to assess what food each man actually received was to be present at his unit and to observe this directly. This was impossible under the conditions of the investigation. Eighty-four of the 124 patients were meat eaters but, as has already been said, many of the troops received only negligible amounts of flesh foods in the jungle areas

Malaria—The majority of the patients stated that they had had malaria in the past, but their statements were of little value. In 51 cases of the 124 examined, the hospital records showed that malaria parasites had been found in the blood at some time during the present illness. Twenty-five were B T, 21 were M T, 5 had had both varieties. All but six men stated that they had received suppressive mepacrine regularly

Ancylostomiasis—Eighty-eight of the 124 patients had more than two stool examinations carried out for ova and cysts. Twenty-seven of these, or 30.7 per cent., had ancylostome ova. The findings are summarized in Table IV

TABLE IV
STOOL EXAMINATIONS OF 88 INDIAN TROOPS SUFFERING FROM MARASMUS

Nil abnormal found	44	<i>Balantidium coli</i> only	1
Ancylostome ova only	18	<i>Hymenolepis nana</i>	1
Ascaris "	6	<i>E. histolytica</i> (veg form)	1
Ascaris and ancylostome ova	4	" and ascaris ova	1
<i>Giardia lamblia</i>	2	" trichuris ova	1
Ancylostome and trichuris ova	2	" trichuris and ancylostome	
<i>Entamoeba histolytica</i> cysts	1	ova	1
Bacillary exudate	1	Ascaris ova and tapeworm segments	1
" , and ancylostome ova	1	Bac exudate, ancylostome ova and <i>Bal</i>	
" , , ascaris ova	1	<i>coli</i>	1

Dysentery—There was nothing in the history of these men to suggest that either bacillary or amoebic dysentery played a part in the onset of their illness

was pale, frothy and bulky like that of classical sprue, other times the motions were merely watery. Microscopically, fatty acid crystals and fat cells were occasionally seen, but they were not present to any great extent. Sometimes undigested food was present without evidence of an increase of fat, and frequently there was no microscopic abnormality. Blood and mucus were not features of the stool. Some patients had anaemia without diarrhoea.

Glossitis—Varying degrees of glossitis were present, there did not appear to be any relationship between the extent of the glossitis and the severity of the anaemia. Some cases had glossitis without diarrhoea. The most severe form seen was an acutely inflamed red tongue, sometimes showing fissuring. It was found impossible to differentiate between a magenta coloured tongue and a scarlet tongue.

Gingivitis—This was equally common amongst men who were not suffering from the syndrome under consideration.

HAEMATOLOGY

Most of the cases described above were suffering from anaemia. In the great majority of cases, the blood film showed macrocytosis, anisocytosis, ovalocytosis, and poikilocytosis. In the more severe cases, normoblasts and megaloblasts were present in the peripheral blood. For reasons already given, the haematological findings for only 27 cases are given in full, in Table VI, but it was possible to establish accurately that in 101 of the cases the anaemia was macrocytic, in 22 orthocytic, and in only 1, microcytic.

It will be seen from Table VI that, according to the findings of the absolute indices, the distribution of the types of anaemia in the cases under consideration was as follows: orthochromic macrocytic, 17; hypochromic macrocytic, 8; hypochromic orthocytic, 2.

It will be seen, further, that it was usual but not invariable to find megaloblasts in the bone marrow of these patients. These cells were indistinguishable from the megaloblasts that one finds in Addisonian pernicious anaemia, except that haemoglobinization of the intermediate megaloblasts was less advanced than in the latter condition. Recently made marrow films of pernicious anaemia patients were available for comparison during the course of the investigation. Sternal puncture was performed in 96 of the cases, and megaloblasts were found in 62 of them. Malaria parasites were never found in the bone marrow when they were not present in the peripheral blood. There was no shift to the right in the Arneth count, and Table VI shows that the reticulocytes varied from less than 1 per cent to 8.25 per cent.

The haematological findings of another case seen during the course of this investigation, but not suffering from symptoms of the type described in the series under consideration, are included in Table VI (Case 28). This was an Indian Sepoy, aged 22, who complained of weakness, giddiness, and anorexia, but who had never had any trouble with his bowels. The stools appeared normal in every way. As far as could be ascertained, his diet had been satisfactory, although he did not eat meat. He had been stationed east of the Brahmaputra, but only in a base area, where supplies had been satisfactory. He

TABLE VI.
HAEMATOLOGICAL FINDINGS (BLOOD AND MARROW) OF 25 PATIENTS.
Cases 1-27 were suffering from the malarious syndrome

Case number		1	2	3	4	5	6	7
BLOOD	Hæmoglobin (G per cent)	8	2.8	3.5	3	4.0	5.1	5.1
	Red cell count (mill.)	0.833	1.191	1.033	844	1.133	1.023	1.110
	White cell count	3,100	3,600	3,800	1,200	2,400	4,800	2,100
	Polymorphs per cent	—	45	34	80	37	64	0
	Lymphocytes	0	25	3	3	40	20	—
	Monocytes	1	—	3	—	1	—	3
	Eosinophil	—	—	—	—	—	4	—
	Basophil	—	—	—	—	—	—	—
	P.C.V. per cent.	11.0	0.1	10.7	12.0	—	14.0	16.2
	M.C.H. (??)	34.7	—	31	4.8	2.1	40.0	34.2
	M.C.H.C. per cent	—	31.9	3.7	30.8	—	34.0	31.3
	M.C.V. (cμ)	131.7	82.4	104.0	134.0	—	142.7	113.0
	Reticulocytes per cent	1	—	2	1	1.5	2.75	2.0
MARROW	Neutrophils	—	3	3	—	1.0	0.5	—
	Myelocytes	—	—	—	—	—	—	—
	Metamyelocytes	16.3	0.5	3.3	1.3	0.0	7.25	4.0
	Polymorphs	20.25	44.3	40.0	31.5	25.3	29.25	1.75
	Eosinophils	—	—	—	—	—	—	—
	Myelocytes	0.3	—	0.75	—	1.0	—	1.5
	Polymorphs	0	1.5	8.75	0.75	3.0	1.0	7.5
	Basophils	—	—	—	—	—	—	—
	Myelocytes	—	—	—	—	—	—	—
	Polymorphs	—	—	—	—	—	—	—
	Pre-mycocytes	—	1.3	0.3	—	0.25	—	—
	Myeloblasts	—	3	0.0	1	—	—	—
	Lymphocytes	3.75	3.3	0	—	3.25	4.75	5
	Plasma cells	—	0.5	—	—	—	—	—
	Monocytes	—	—	—	—	—	—	—
	Megakaryocytes	—	1	—	—	—	—	—
	Pre-erythroblasts	1.75	7.5	2.5	—	3.75	0.5	0.5
	Early normoblasts	—	—	—	—	—	—	—
	Normal	—	1.3	1.3	3.0	0	5	1.0
	Hypochromic	5	5	4.0	—	—	—	1.25
	Early megaloblasts	1.3	3	0.5	—	3.25	2.5	—
	Intermediate normoblasts	—	—	—	—	—	—	—
	Normal	3.75	4	10.3	4.5	—	1.0	12.0
	Hypochromic	3.3	0.5	1.75	—	1.0	—	—
	Intermediate megaloblasts	10	1	1.75	3	9.0	20.5	0
	Late normoblasts	—	—	—	—	—	—	—
	Normal	13.0	3	10.3	—	0.5	—	1.25
	Hypochromic	4.75	0.5	0.25	—	0.5	—	3.5
	Late megaloblasts	0.5	3.0	3.0	1.5	4.25	31.5	0.25

These two cases had hæmaturia reading carried out a few days later and were shown to be orthochromic and macrocytic

TABLE VI—continued.

Case number		13	10	17	18	10	20	21
BUNO	Haemoglobin (G per cent.)	8.0	8.1	8.4	8.5	8.3	8.6	8
	Red cell count (mill.)	2.243	2.1	2.25	2.43	2.663	2.05	2.02
	White cell count	9,100	~300	3,200	3,500	4,600	4,300	4,500
	Polymorphs per cent.	23	48	31	56	23	35	56
	Lymphocytes	56	61	40	38	36	78	31
	Monocytes	—	1	4	2	1	4	4
	Eosinophils	0	2	4	3	6	10	6
	Basophils	—	—	—	—	—	—	—
	P.C.V. per cent.	76.2	23.3	4.9	31.0	24.7	—	29.0
	M.C.H. (γ)	35.4	34.7	33.3	33.8	31.9	41.2	21.3
	M.C.H.C. per cent.	30.3	34.9	33.7	27.4	29.8	—	39.3
	M.C.V. (μ)	116.7	185.4	104.6	122.3	107.7	—	90.1
	Reticulocytes per cent.	0.75	<1	<1	<1	<1	8.25	<1
MUSKOW	Neutrophils	—	—	—	—	—	—	—
	Myelocytes	0.3	1.8	—	—	1.25	0	1.75
	Metamyelocytes	13.8	10.23	0.75	1.75	7.5	27.0	24.8
	Polymorphs	31.0	32.8	20.0	7.5	4.75	47.8	37.5
	Eosinophils	—	—	—	—	—	—	—
	Myelocytes	0.25	—	0.8	0.25	—	0.8	2.5
	Polymorphs	3.0	0.75	2.25	1.25	0.5	0.25	5.8
	Basophils	—	—	—	—	—	—	—
	Myelocytes	—	—	—	—	—	—	0.25
	Polymorphs	1.0	—	—	—	—	—	0.75
	Preerythrocytes	0.75	0.75	0.25	0.5	0.75	—	1.0
	Megakaryocytes	1.0	0	7.8	2.3	0.75	—	1.25
	Lymphocytes	0.25	—	—	—	0.25	—	2.0
	Plasma cells	—	—	0.75	—	—	—	0.25
	Monocytes	—	—	—	—	—	—	1.8
	Megakaryocytes	—	—	—	—	—	—	0.5
	Pre-erythroblasts	5.0	1.8	—	—	—	—	1.8
	Early normoblasts	—	—	—	—	—	—	—
	Normal	0.5	2.8	10.8	10.75	—	1.5	3.25
	Hypochromic	0.5	3.0	—	—	1.0	—	—
	Early megakaryoblasts	—	1.5	—	—	—	—	—
	Intermediate normoblasts	—	—	—	—	—	—	—
	Normal	10.0	0.5	23.8	20.25	—	0.8	4.75
	Hypochromic	1.25	3.0	—	—	1.0	—	—
	Intermediate megakaryoblasts	—	5.0	1.25	1.5	—	—	—
	Late normoblasts	—	—	—	—	—	—	—
	Normal	18.3	0.8	21.5	20.25	—	10.75	23
	Hypochromic	0.8	22.75	—	—	41.25	—	1.8
	Late megakaryoblasts	0.5	4.25	7.5	1.8	—	—	—

These two cases had haematocrit readings carried out a few days later and were shown to be orthochromic and anisocytic.

gave history of ten attacks of malaria there was no clinical evidence of vitamin deficiency. The spleen was very much enlarged, and the liver was palpable. Hookworm ova were present in the stool. It will be seen that the mean corpuscular volume was very high in this case, and that megaloblastosis was very marked in the marrow. His serum, like that of several of the patients suffering from the marasmus syndrome, was icteric, although no malaria parasites were present in the blood. He had persistent reticulocytosis, the count on one occasion being 25 per cent. He was given 8 c.c. of crude liver extract intramuscularly on 2 successive days, and then 4 c.c. on the third day, suppressive mepacrine being stopped for month prior to the injections. On the third day parasites of benign tertian malaria were seen in large numbers in the peripheral blood. A course of quinine, mepacrine and pamaquin was given without further liver injections and the patient's blood rapidly returned to level approaching normality.

THERAPEUTIC TESTS.

The previous hospital records of all cases believed to be suffering from the marasmus syndrome were studied, and it was evident that many cases of megaloblastic anaemia were resistant to any form of therapy. Many had been given courses of quinine or mepacrine without haematological improvement. Others had been given crude liver extract by injection or an autolysed yeast preparation by mouth without significant improvement in the general condition, the number of stools, or the blood picture, even when this therapy was preceded by the administration of quinine.

Twelve patients who had recently been given crude liver extract by intramuscular injection (in one case oral live extract by mouth) had bone marrow examinations carried out. Table VII shows that in most of these significant degrees of megaloblastosis persisted.

Eight typical cases of the marasmus syndrome with abdominal symptoms, diarrhoea, anaemia, and megaloblastic marrow were selected for controlled live therapy. Suppressant mepacrine was stopped on the first day of the investigation, and, after control period, during which very high caloric diet was given, injections of crude liver extract were commenced. The results were as shown in Table VIII.

It will be seen that case T1 improved with diet alone and that this improvement was maintained or augmented with liver therapy. In case T3 there was definite response to liver therapy—the reticulocytes rose to 27 per cent. on the ninth day of the injection. In no other case was definite reticulocyte response obtained, nor was there rise in the level of the red cells or haemoglobin.

It was found, however, that the parasites of benign tertian malaria appeared in the blood of six of the cases, usually after liver therapy—in one case before the treatment was begun. Five control cases of men coming from the same area, and suffering from similar clinical features had suppressant mepacrine stopped for periods of 3 weeks, no liver injections were given, and malaria did not occur. A further five cases suffering from hypochromic microcytic anaemia, with no evidence of megaloblastosis in the marrow were selected for crude liver injections after control period of fortnight, mepacrine again being stopped for the period of the test. One case developed B.T. malaria during the control period, one did not develop malaria, and the remaining three developed it following the liver injections.

For reasons of service, it was not possible to follow this up on an adequate number of cases or controls.

This test was carried out in an area where fresh infection with malaria was unlikely during the period of the test. Every precaution was taken to ensure that the patients were not bitten by mosquitoes while under observation.

TABLE VII

TO SHOW THE PERSISTENCE OF MEGALOBLASTOSIS IN BONE MARROW FOLLOWING LIVER THERAPY

Case	Days of liver therapy	Total amount of liver given by 1 m l during this period	Day of sternal puncture from commencement of liver therapy	Red cells in peripheral blood on day of sternal puncture (per c.mm)	Megaloblasts as percentage of total red cell precursors in marrow		
					Early	Intermed	Late
A	1- 9	18 c c	14	1,310,000	2 4	8 8	2 0
B	1- 5	12 "	13	2,820,000	—	—	1 4
C	1-17	14 "	26	1,320,000	1 7	3 0	—
D	1-11	24 "	23	2,000,000			
	1-18	Oral liver extract 1 oz t i d			0 9	10 4	4 7
E	1- 6	12 c.c.	20	840,000	1 5	11 7	12 7
F	1-11	12 "	30	2,430,000	—	5 5	2 2
G	1- 7	16 "	27	2,830,000	—	7 0	2 3
H	1-10	12 "	31	1,480,000	5 1	14 0	2 5
I	1-12	12 "	38	1,500,000	—	8 3	1 0
J	1- 6	12 "	33	1,430,000	3 4	17 9	14 6
K	1- 9	8 "	38	2,100,000	3 7	14 8	7 4
L	1-11	Oral liver extract 1 oz t.i d	12	1,290 000	0 5	1 7	3 2

When malaria did develop, it was treated with mepacrine in a dosage of 0.6 gramme daily for 2 days, 0.3 gramme daily for 5 days, and 0.1 gramme daily thereafter. This treatment caused no improvement in the haemoglobin or red cell levels.

At the time of the investigation it was found impossible to obtain supplies of proteolysed liver.

DISCUSSION

This account deals with a very small number of cases of a syndrome that affected large numbers of Indian troops stationed east of the Brahmaputra river. It did not affect either British or African troops stationed in the same areas to any great extent, and it was not seen in Indian troops from a similar population stationed in other parts of India. The cause was uncertain, but the general features of the condition were very similar to those of sprue in Europeans. It has long been said that sprue is uncommon amongst Indians, but recently several authors have referred to a sprue-like condition occurring in natives of India. Cook (1944) has described such a syndrome as he had seen it in Gujerat. Unfortunately it was not possible to carry out any more than the simplest

of investigations under the conditions of the field survey described in the present paper it was impossible to carry out stool fat estimations. Some patients had the features of avitaminosis of the B complex, without having either diarrhoea or anaemia, but it is possible that accurate fat balance tests carried out as described by COOKE, ELKES *et al.* (1948) would have given evidence of impaired fat absorption. On the other hand primary malnutrition in itself may lead to diarrhoea.

The blood picture in the present series is very similar to that of nutritional macrocytic anaemia (formerly called tropical macrocytic anaemia), a condition described by WILLS and MEHTA (1930), and by WILLS (1931 and 1934). The condition was found to respond to autolysed yeast preparations by mouth, or to crude liver extract by injection, but not to injections of refined liver extract preparations. FAIRLEY *et al.* (1938) describing a similar condition amongst refugees in Macedonia, have divided cases of nutritional macrocytic anaemia into two subgroups—non-haemolytic and haemolytic. The haemolytic element was attributed to malarial infection. TALIAFERRO and MULLIGAN (1937) have referred to the possible phagocytosis of normal red cells by the hypertrophic reticulo-endothelial system in monkey malaria—this would increase the hyperplastic tendency of the bone marrow which is already showing red cell proliferation to compensate for the destruction of red cells by malaria parasites. NAPIER (1939) has pointed out that hyperplasia of the marrow from this cause makes it necessary for an increased supply of haemopoietic factors to be available, and if there is already a tendency towards megaloblastic anaemia from deficiency of Wills's factor the malaria will thus cause a relatively greater deficiency and increase the megaloblastic trend. TROWELL (1947) has described a dimorphic form of anaemia associated with malnutrition, hookworm infestation, and malaria in Uganda, and has shown that, provided 5 days treatment with quinine is first given to combat chronic malaria, a double reticulocyte response will be obtained to crude liver extract injections and iron by mouth. His views have recently undergone modification (TROWELL, 1947). HIRSH and ISLAQ (1945) working with North West Indian troops, have pointed out the extent of a hypochromic form of anaemia amongst such men—this was thought to be due to the fact that the greater part of the dietary iron was not being assimilated.

It is evident that the anaemia described in the present series of cases is of a very complex type. Possibly years of primary malnutrition prior to joining the Services played a part in many cases, and was aggravated more recently by supply difficulties during jungle fighting. Thus there might be a deficiency of Wills's factor and conceivably of extrinsic factor which would give a tendency to megaloblastosis. In addition, there was a sprue like condition which itself might induce a megaloblastic form of anaemia due to malabsorption of the liver factor produced by the interaction of Castle's extrinsic and intrinsic factors, or of folic acid conjugate (DAVIDSON and GIRDWOOD 1947). To what extent prolonged low intake of protein of high biological value plays a part is unknown.

Case	Anemia type	Red cell count (mills) and day of investigation						Haemoglobin (grammes per 100 ml) and day of investigation						Days B 1 st parasites found	Treatment
T1	Orthochromic Macrocytic	1 2 08	1 6 2 45	1 11 3 14	1 10 3 12	1 9 3 11	1 8 3 10	8 6 8 7	10 1 10 1	15 10 8	19 11 4	23 12 4	11 13 2	21 35 12 4	Liver inj (8 cc) Days 14, 15 , (4 cc) " 16-21 Mepacrine course, 22 on
T2	Hypochromic Orthocytic	1 1 14	1 4 2 10	1 12 2 00	1 16 2 00	1 20 1 10	1 24 1 00	32 2 08	1 4 4 7	10 1 2 4	20 1 8	21 2 9	28 5 0	3 32 5 1	Mepacrine course, 7-10 Liver inj (5 cc), 17, 18 " (4 cc) " 19-24*
T3	Orthochromic Macrocytic	1 1 37	1 9 1 30	1 13 1 09	1 17 1 21	1 21 1 05	1 25 1 00	34 1 27	1 9 5 9	17 1 6	21 5 4	24 6 1	29 6 5	11 5 2	" (8 cc) 14, 15 Mepacrine course 15 on
T4	Orthochromic Macrocytic	1 1 37	1 5 1 17	1 13 1 22	1 17 1 21	1 21 1 22	1 25 1 80	13 1 50	1 5 5 2	13 5 1	17 5 3	21 7 2	29 7 1	25 6 5	Liver inj (8 cc) " 15, 16 (4 cc) " 17-21 Mepacrine course 25 on
T5	Orthochromic Macrocytic	1 1 44	1 6 1 32	1 10 1 30	1 14 1 00	1 18 1 00	1 22 1 00	33 2 51	1 6 5 5	10 5 6	17 6 3	21 8 0	25 9 4	Nil 33 10 0	Liver inj (8 cc) " 14, 15 (4 cc) " 16-23
T6	Orthochromic Macrocytic	1 1 39	1 5 1 30	1 9 1 35	1 11 1 16	1 15 1 11	1 21 1 10	25 1 44	1 5 7 0	9 6 1	13 5 5	16 5 6	21 6 7	20	" (8 cc) 10, 17 (4 cc) 18-20 Mepacrine course 21 on
T7	Hypochromic Orthocytic	1 3 80	1 5 3 51	1 9 3 87	1 13 3 02	1 17 3 02	1 21 3 70	25 1 80	1 5 9 7	9 9 1	14 9 5	19 8 5	25 8 8	18	Liver inj (8 cc) 15, 16 (4 cc) 17, 18 Mepacrine course 19 on
T8	Orthochromic Macrocytic	1 2 60	1 5 2 41	1 9 2 10	1 13 2 37	1 17 2 41	1 21 2 37	25 2 42	1 5 10 8	9 10 0	14 10 0	17 10 1	21 10 2	Nil	Liver inj (8 cc) 16, 17 (4 cc) 18-25

* This patient was given a transfusion of 2 pints of blood on the 24th day. All liver extract was crude and was given by the intramuscular route.

Malarial infection, when present in men suffering from such a deficiency syndrome, might add a haemolytic element, and thus increase the megaloblastosis in the manner described above. It is likely that the majority of the men seen in connection with this investigation were suffering from chronic malaria which had been suppressed by mepacrine. The figures for the haemoglobin level of 500 troops given in Table I make it obvious that this in itself would not produce anaemia since those men, also, had been taking suppressive mepacrine for long periods, and had been fighting in the same malaria-infected jungles. It was noted that the serum was icteric in several of the cases suffering from the macrocytic form of anaemia, and that this was usually associated with a raised reticulocyte count. However many of the men suffering from chronic malaria and macrocytic anaemia did not show a reticulocytosis or other evidence of haemolysis.

The development of an acute attack of malaria following injections of crude liver extract was thought to be due to the liver acting as a form of shock therapy producing clinical evidence of a latent infection. In some cases such evidence was obtained when suppressive mepacrine was stopped although liver was not given.

In addition to the above factors tending to produce megaloblastosis, it is likely that there was an iron deficiency of the type described by ILLERS and ISLAU (1945) operating in many of these men: this would be augmented by the sprue-like state, which would make the absorption of iron even less satisfactory and to add to this deficiency there was the effect of infestation with ancylostomes.

The severity of the megaloblastic anaemia was greater in this series of cases than is generally present in Europeans suffering from sprue and for this reason primary malnutrition is considered to be an important factor in the production of the haematological picture. Moreover as has already been said British and African troops stationed in the same areas were not similarly affected. It is to be noted that ILLERS *et al.* did not find that macrocytic anaemia was a problem amongst North-West Indian soldiers (1945), or amongst recruits in the south of India (1946). When these troops went east of the Brahmaputra river they had to carry out an increased amount of work, giving greater demands for nutritional factors at a time when supply difficulties were acute. The Indian troops were at a greater disadvantage than other men on account of their religious prejudices about foodstuffs. Other factors encountered in the affected area of country were the sprue like disease and, possibly, a more severe form of malarial infection.

Recently synthetic folic acid (pteroylglutamic acid) has been shown to be effective in the treatment of megaloblastic anaemia, including the form associated with malnutrition (SPIES *et al.* 1945), and in sprue (LOPEZ, SPIES *et al.* 1946). Moreover in the latter condition marked improvement in the clinical state has occurred when synthetic pteroylglutamic acid has been given. It has been

suggested by BERRY and SPIES (1946) that in health free folic acid is derived from folic acid conjugates which are present in the food and are stored in the body. Experiments by WELCH *et al* (1946) support this suggestion, and a simple explanation would be that the active principle in refined liver extracts acts by causing the liberation of free folic acid from conjugated forms which are themselves haemopoietically inactive. This is discussed by WELCH (1947) and arguments against this simple explanation are given.

It is likely that clinical and haematological improvement would have occurred in these patients had folic acid been available for administration. It is of interest that the clinical features of the syndrome are similar in many ways to those of folic acid deficiency in the monkey (RINEHART and GREENBERG, 1947). It is possible that Wills's factor is, in fact, folic acid.

SUMMARY

1 An account is given of a syndrome of diarrhoea, wasting, avitaminosis, and anaemia, confined almost entirely to Indian other ranks stationed east of the Brahmaputra river.

2 The condition was similar in its clinical features to sprue as it occurs in Europeans.

3 An account is given of the extent to which anaemia was a problem in a hospital population of 500 unselected Indian other ranks in transit from the forward areas.

4 An account is given of the degree of anaemia in Indian other ranks admitted to a special anaemia centre during a period of 28 days.

5 The type of anaemia present in 27 cases of the marasmus syndrome is detailed.

6 An account is given of the poor response to parenteral administration of crude liver extract in several of these cases.

7 The factors tending to produce anaemia in the cases are discussed.

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have accounted for but a very small proportion of the total incidence in the Command. This may be illustrated by the totals for 1942, during which 288 out of 303 cases admitted to hospitals were in Harrar Dire Dawa, Diego Suarez and Mogadishu, and for 1944 in which there were 248 cases admitted to hospitals at Diego Suarez and 40 elsewhere. In 1945 after effective control of *Aedes* at Diego Suarez had been achieved, there were by contrast only 3 cases in all, admitted to hospitals up to the end of September.

However much allowance may be made for failure to diagnose atypical examples of the disease, it is clear therefore that the conditions for the production of a high degree of dengue transmission are for the most part absent in East Africa. Whether this is mainly to be attributed to the absence of virus or to insufficient prevalence of vectors, cannot be determined with any certainty but in the opinion of the writers it is likely that the latter explanation is the more probable. It should be noted that there was no difference between the composition of the military populations in those places in which there was a heavy incidence of dengue, and elsewhere.

Where dengue was prevalent a much higher proportion of European cases than African was found. For example, it is known that the European incidence in units stationed at the port of Diego in 1943 was over 100 per cent. for the season whereas the rate for all races was only 12.3 per cent. for the same period. The explanation is, of course, to be found partly in the greater difficulty of elucidating the cause of a mild illness in an African, and partly in the difficulty of seeing a rash on a dark skin. The chief reason for calling attention to this difference between races is the indication which it gives that the total incidence was very much higher during epidemics than the rates given would indicate.

COURSE OF EPIDEMICS.

The first major outbreak was at Mogadishu. This began soon after our occupation of this town in March, 1941. The first clear indication of a high dengue incidence was given in May when 72 cases were admitted to hospitals and many more occurred. Lesser numbers of cases continued for the rest of the year and there was a second peak of incidence during May 1942, although not so great as in the previous year. As a measure against yellow fever *Aedes* control was begun in July 1942, and from then onwards the occurrence of dengue became rarer and rarer with the result that in 1943 and 1944 there was no seasonal occurrence. During the Italian regime, when no *Aedes* control seems to have been attempted, the epidemic occurrence of dengue was expected once or twice annually.

The second major outbreak was at Diego Suarez, in June, 1942, again shortly after the British occupation of the port. Many cases were diagnosed as sandfly fever and in view of later experience and the complete absence of *Phlebotomus* from the area, these were included in the dengue figures. In

January, 1943, cases again began to occur, and the number increased to a peak in April, falling away again to disappear by the middle of June. As a result of this experience, and the dislocation which it caused among units of all services, an *Aedes* control was established towards the end of 1943. Owing to various difficulties, it was not possible to make this control as effective as had been desired, but it was sufficient to reduce greatly the expected epidemic, which was cut short during the last week in March. Efforts at control were continued, and during the 1945 season only 3 cases were diagnosed. It should be noted that there was in each of these years a change over of at least 80 per cent in the service personnel at risk. It may also be remarked that, as at Mogadishu, the French and other residents accepted these annual outbreaks of dengue as inevitable, often referring to the disease as *la grippe*.

The duration of the epidemic periods at Diego Suarez was no doubt to some extent determined by the availability of susceptible Europeans, in 1942 the whole military population was presumably susceptible. There was a steady change over of military units during the latter part of 1942 and early 1943, and it is substantially true that by the end of June, 1943, nearly all susceptible European soldiers present at that time had been attacked.

By the beginning of 1944 an almost wholly susceptible population had again accumulated, and in June and July there was a further large influx of those who had not previously been attacked. In spite of this, there was not any incidence of dengue in the latter part of the year.

THE CLINICAL ASPECTS OF CASES IN THE COMORO ISLANDS AND DIEGO SUAREZ

The onset was in all cases sudden with a rigor in 50 per cent of cases, and a temperature rapidly reaching 103°F to 104°F . This initial rise of temperature was accompanied by moderate prostration and severe frontal or post orbital headache. Coincident with these, increasing suffusion of the face occurred, with congestion of the conjunctivae and photophobia of varying degree. Severe pains, most marked in the lumbar region, were present in most cases. The severe bone and limb pains which have given to dengue the name of "break-bone" fever, were not seen. The tongue varied: it was most frequently covered with a dense white fur except along the margin and tip. In about 50 per cent of cases it remained clean. Abdominal discomfort with retching was seen in about 10 per cent of the cases, during the 2nd, 3rd and 4th days.

In nearly half the European cases a rash appeared between the 2nd and 4th days. This was quite distinct from the initial suffusion which is sometimes erroneously called an initial rash. The rash proper was rubeoloid on a erythematous background in all fully developed cases. This fully developed rash was seen in about 20 per cent of European cases. The chief sites of this rash were over the limbs and trunk, the front of the chest and abdomen, and

have accounted for but a very small proportion of the total incidence in the Command. This may be illustrated by the totals for 1942, during which 238 out of 303 cases admitted to hospitals were in Harar Dire Dawa, Diego Suarez and Mogadishu, and for 1944 in which there were 246 cases admitted to hospitals at Diego Suarez and 40 elsewhere. In 1945 after effective control of *Aedes* at Diego Suarez had been achieved, there were by contrast only 3 cases in all, admitted to hospitals up to the end of September.

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exceeding 101° F. In these cases no other signs or symptoms were observed, but from 16 to 21 days later a typical attack often followed, though in none was the severity very acute. There were 37 such cases recorded. Whether the two attacks were connected is unknown, but the apparent association is recorded.

In almost all cases the pulse rose to about 100 per minute with the initial rise in temperature, but fell to 70 and below after 24 hours. This bradycardia persisted throughout the course of the disease and was prolonged into the 2nd week of convalescence. In the few cases where the blood pressure was recorded, the pressure was normal or somewhat lowered. The lowest recording was a systolic pressure of 110 mm Hg.

White blood counts were done in a few cases. In Diego Suarez these showed a definite leucopenia on the 4th day—the count being down to (approximately) 4,000 per c mm. This decrease was due to a drop in the number of polymorphonuclears—the neutrophils in particular. The absolute count of other cells remained within normal limits. In the Dzsaudsı (Comoro) cases the drop was chiefly due to diminution of lymphocytes.

No treatment is specific and no drug was found to have any effect on the duration of the disease. The most distressing symptoms were sleeplessness, restlessness from general bodily aches, headache and vomiting. Codein, opium, and aspirin were found to be the most effective drugs in keeping the patient comfortable.

The disease is amazingly variable in its clinical manifestations, with no predominant symptom recurring as a characteristic, each symptom apparently taking its turn as the dominant feature. The above outline is not intended as a description of the disease but rather as a composite picture of the cases as we saw them during these epidemics.

AEDES PREVALENCE AND DENGUE

The main interest in the records of *Aedes* is in the contrast of the indices at Mogadishu and Diego at the times at which dengue disappeared. The first ascertainment of the *Aedes* index at Mogadishu was in February, 1942, and at this time it was about 27 per cent. It would be a fair assumption that during the dengue epidemic of 1941 the index was even higher. Following the reduction in the *Aedes* index by control measures during the latter part of 1942, dengue was substantially abolished, and to a negligible level when the index dropped below 10 per cent. It may well be that the occasional cases recorded in 1943 and 1944 were acquired outside Mogadishu, but it is at least certain that no epidemic occurred after the *Aedes* index had fallen below 10 per cent.

At Diego Suarez, on the other hand, dengue disappeared only when the *Aedes* index was less than 2 per cent. This relationship was shown both during the natural seasonal fall occurring in 1944, and as a result of the reduction in the *Aedes* index produced by control measures during 1945. The first survey

the inner aspect of the arms and thighs. In many cases the rash was of short duration, in two cases being very evanescent and only of about 1 hour's duration. This characteristic of the exanthematous eruption suggests that it is much more common than is recorded, only the more persistent instances being noted. This is borne out by the fact that the percentage of rashes during the latter part of the epidemic was three times greater than that recorded over an equivalent period at the beginning. It is suggested that the reason is that it was more particularly sought and enquired about by both patients and attendants.

The mucous surfaces are also subject to an exanthem which was frequently seen as a dusky redness of the fauces and palate with, in about 10 per cent. of cases, a vesicular stippling of the soft palate. This was usually accompanied by some weakening of the voice and huskiness, but without soreness or irritation. This eruption cleared up with the final fall in temperature. With the fall in temperature the exanthem also faded, to be followed in most cases by a very fine desquamation. This is difficult to see on a European but is very evident on the dark African skin which gives the appearance of having received a slight sprinkling of fine powder.

Following this desquamation itching of the palms and soles was intense in a few cases and present in most. Desquamation over other areas was seldom accompanied by itching or irritation. Where it was marked, it caused distress for 48 to 72 hours, mostly through interference with sleep.

During the disease, alterations of taste were common. This is, in our opinion, a very prominent feature of the disease for in several of the very mild and apparently abortive attacks it was the only symptom of which the patient complained. The most common description was that of an "astringent metallic taste."

In some 25 per cent. of our cases lymphatic enlargement was found. In every case only a single gland or group of glands was found and in no case was generalized lymphadenitis seen. The most commonly affected were in order of frequency inguinal, epitrochlear and cervical, and as enquiry showed that the most frequent sites of mosquito bites were the knees (under desks), hands, and the back of the neck, there may be some correlation between the site of the infective bite or bites and the position of lymphatic gland enlargement.

The temperature is usually described as of "saddle-back" type, but our experience of these epidemics demonstrated as in other epidemics, that the type of temperature was one of the most variable features of the disease—intermittent remittent and continued type temperatures being shown with almost equal frequency. Typical saddle-back charts formed only about 10 per cent. of the total. The most constant feature of the temperature chart was the fall by crisis on the 6th day. In fully developed cases there was no exception to this rule.

In a number of instances there was what appeared to be an abortive attack, characterised by malaise and fever for 24 to 72 hours, the temperature never

THE TOLERANCE OF THE METACYCLIC AND FLAGELLATE FORMS OF *LEPTOMONAS CTENOCEPHALI* FANTHAM TO VARIATIONS OF HUMIDITY AND SALINITY

BY

ALFRED J GIBBS *

The present investigation represents a continuation of previous work (GIBBS, 1947), which dealt particularly with the disseminated forms of *Leptomonas ctenocephali*, parasitic in the digestive tract of the dog flea, *Ctenocephalus canis*. It was then noted that the resistant, leishmania-like forms in the faeces of infected fleas remained viable for a period of up to 2 months, and during that time would develop within an hour into active leptomonads if immersed in physiological saline. Similar forms were also obtained directly from the hind-gut and rectum by dissection, and after being dried for some hours, developed in saline in a manner identical with those found in the faeces. In contrast the leptomonads were found to be only slightly resistant to desiccation, they could be dried for only 15 minutes without loss of viability.

The term "resistant body" was previously employed with reference to the leishmania-like forms found in the faeces, but since the envelopment of the bodies by the faeces is an essential factor in their surviving over long periods, it appears doubtful whether the use of the term is justified. The term "metacyclic form" is considered more applicable, and will be used in the present work.

REACTION TO DRYING AND VARIATIONS OF HUMIDITY

The flea's faeces are found on heavily infested dogs in the form of hard dark granules, particularly in the region of the animal's back. In a previous paper it was suggested that the rounded leishmanial bodies found in the rectum and hind-gut of the flea, and which are morphologically identical with the

* It is desired to express thanks to Dr ANDREW ROBERTSON (formerly lecturer, Department of Protozoology, London School of Hygiene and Tropical Medicine), and to Professor J T IRVING (Department of Physiology, University of Cape Town) for much valuable assistance.

in July 1943 showed an *Aedes* index of 7.2 per cent. and it may again be assumed that during the epidemics of 1942 and 1943 the index was much higher than this. It is believed that a great reduction in domestic *Aedes* breeding was achieved during the last 4 months of 1943 and the early months of 1944 as compared with the previous 12 months, and that this reduction was responsible for the lesser incidence of dengue, but the control still failed (owing to the nature of the work involved) to complete the eradication of breeding in the many roof gutters, three or four stories high, and in a multitude of tree holes, found both in street avenues and elsewhere. Such breeding foci were not reflected in the *Aedes* index, but were, it is believed, responsible for the residual dengue during the rains of 1944. The completion of the treatment of the tree holes in the latter part of 1944, was followed by the virtual disappearance of dengue in 1945.

At Dire Dawa there were twenty-five cases in July 1941—seven in August—one to five in the following months. During this period the *Aedes* index varied from 3.0 to 3.5 per cent. in the European part of the town, but ranged round a very approximate figure of 50 per cent. in the adjacent African town which was separated only by the breadth of a dry river bed from the European area where troops were quartered.

SUMMARY

1. A description of the occurrence of dengue in the East African Command, during the years 1941-45, is given.

2. Dengue was, for the greater part, confined to a few localities in which *Aedes* mosquitoes were specially prevalent.

3. A description of the clinical features of the epidemics in Madagascar is given.

4. Dengue was almost completely eradicated by *Aedes* control in the two major epidemic localities—but while in the one case it disappeared when the *Aedes* index had fallen below 10 per cent. in the other case it disappeared only when this index had fallen below 2 per cent.

5. The *Aedes* index requires interpretation before its significance as a measure of the probability of transmission of *Aedes* borne disease can be accepted.

ALFRED J GIBBS

It is realized that the salinities given are only approximate since in all cases the salt content of the faeces increases the concentration. Furthermore, when the material has been dissolved in saline, dried and re-dissolved in the same solution, there will obviously be an increased salt concentration. In this particular experiment water was tried for the second dissolving, but caused numbers of the metacyclic forms to disintegrate before it reached the necessary concentration.

SUMMARY

1 The metacyclic forms remain viable over long periods only when enveloped in the dry granules of faeces

2 A degree of humidity sufficient to reduce the faecal granules to a very soft state will, if maintained for about 3 days, result in the death of the organisms

3 Development of the metacyclic forms will take place only in salt concentrations between 0.25 per cent and 2.3 per cent. Viability is not immediately affected by any further degree of salt concentration. Development will proceed following an appropriate dilution of the liquid medium

4 Leptomonads become non-motile in salt concentrations of 4 per cent and over, but will revive if the salinity is reduced to below 4 per cent within a period which varies according to the concentration employed

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effect on the organisms. Their appearance in normal saline is characteristic, and it is possible, therefore, to cease the addition of water at the point where their usual appearance is resumed.

The effect of hypertonic solution on active leptomonads, obtained from the flea by dissection, was also studied. It was found that the parasites immediately became non-motile in saline strengths of 4 per cent. and over. They appeared to be dead, although they did not assume the granular vacuolated appearance of dead leptomonads. They could be kept in this condition for 2 hours in a saturated solution and would become motile almost immediately the salt concentration was reduced to normal. The period during which they remain viable in the immotile state is dependent on the strength of the solution. A few flagellates became active after 18 hours in a 4 per cent. solution.

DISCUSSION

The need for the disseminated metacyclic forms to be enveloped and sealed off by the faeces of the flea in order to survive over long periods is of interest, and may be without recorded parallel. The question as to whether or not the bodies are protected by a cyst wall has been discussed by many authors, and WENYON (1926) remarks that in so small an organism absolute proof of the existence of a cyst is difficult to obtain. The observations recorded in the present study especially those on the osmotic reaction of these bodies, point strongly against the existence of a cyst wall.

The experiments on humidity as affecting the enveloped bodies show that development does not begin until the faeces are reduced to a thoroughly liquid condition, but that subjection to very humid conditions over long periods, although without dissolving results finally in the death of the organisms. Assuming the accuracy of the hypothesis that viability of the disseminated bodies within the faecal granule is due to the absence of oxygen, it would be expected that in moistening the enveloping faeces and therefore admitting oxygen, metabolism might be stimulated to a point which could not be maintained within the faeces, and which would subsequently result in the death of the enveloped metacyclic bodies.

A certain amount of work has been done relating to the acclimatization of fresh-water protozoa to sea water by a series of gradually increased salinities, but the effects of varying concentrations on the parasitic forms appears to be practically an unexplored field. LOZREN (1909) found that the fresh-water flagellate *Astasia* sp. was possessed of a marked degree of tolerance to high salt concentrations. He states that the organism never remained motile in concentrations above 1.59 per cent., but it was viable after 200 hours in a solution of 3.97 per cent. Although these concentrations are not as high as some used in the present investigation, there is a definite similarity in the effect on the organism.

ALFRED J GIBBS

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SUMMARY

- 1 The metacyclic forms remain viable over long periods only when enveloped in the dry granules of faeces
- 2 A degree of humidity sufficient to reduce the faecal granules to a very soft state will, if maintained for about 3 days, result in the death of the organisms
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- 4 Leptomonads become non-motile in salt concentrations of 4 per cent and over, but will revive if the salinity is reduced to below 4 per cent within a period which varies according to the concentration employed.

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CAPILLARIA HEPATICA

A CASE REPORT

BY
OTTO TIEMANN BROSIUS, M.D., D.T.M. & H., F.A.C.P.,
ESTHER E. THOMAS, B.S.,
AND
BARBARA BROSIUS

A Panamanian Mestizo woman (V.M., aged 28), was admitted to the hospital of the Chiriqui Land Company, Puerto Armuelles, Panama, early in the morning of 2nd November, 1946. Past history is unimportant and negative save for several severe attacks of "paladismo" (malaria), which, needless to say, is endemic in these parts. Marital history reveals that she has been married about 8 years, during which time she bore four children, one of which died at 11 months and another at 13 months, the other two being still alive and well. She does not know the cause of her children's death, but describes each one as having been preceded by fever, nausea, vomiting and terminating in "ataques" (convulsions). There were no abortions or miscarriages. Menses now are regular and quite normal.

Another feature of her marital history is that her husband, although an employee of the agricultural department of the company, is a "mighty hunter," who spends most of his off-duty time in supplying wild meat for his family. This consists chiefly of "gallineta" and "perdis" (both of which bird species belong to the grouse family), "conejo pintada," also called "tipesquite," "guagna" or "guatinaja" in other Latin-American countries, which is an amphibian rodent much resembling a small pig, the flesh of which is considered by many gourmets as the most delicious of all, venison (a very small antelope-

like deer) pavo (tropical wild turkey) and pauju "a bird almost as large as the domestic turkey which, an ornithologist informed one of the authors (O.T.B.) really belongs to the pheasant family. She emphatically denied that she or her family ever ate "caino," a species of wild hog or peccary which wanders in droves through the jungles of Central America. She denied also ever having eaten any form of monkey meat, and assured us that most of the flesh consumed by her or her family was wild meat but when this was unobtainable she frequently prepared beef, usually preferring liver which she fried, to any other cuts. She declared that meat was always cooked well and that no underdone meat was ever eaten.

Present Illness On admission we found the woman suffering from agonizing pains in the epigastric region. She gave a history of having had this pain at short and frequent intervals for about 3 days, and could not associate it with anything in particular that she had eaten. The last meal, however that she had before the onset of this pain consisted chiefly of fried beef liver. Other members of her family had partaken of this same meal, but none of them became ill after it. For 3 days she tried home remedies which she found of no avail and the pains becoming gradually more agonizing she was brought to the hospital in the early hours of the fourth day after the onset of the illness. Such an attack of pain and distress was entirely new and she had never suffered from anything like it before. There was nausea with the pain, but she vomited only once, and the pains were always definitely localized in the region of the pyloric end of the stomach.

Physical Examination Practically everything was found to be negative save in the upper abdomen. The epigastric region, and also the two upper abdominal quadrants, were absolutely rigid and extremely tender. Sudden release of pressure while palpating the two lower quadrants of the abdomen which were comparatively soft and flaccid, was not accompanied by pain. There was no distention with gas. One drachm paregoric was given immediately after the examination, but was vomited about 15 minutes after it had been swallowed. A second dose of paregoric was likewise vomited when administered about half an hour later. Application of a hot water bag also brought not the slightest relief therefore a quarter grain of morphine sulphate was injected subcutaneously. This brought relief for several hours but had to be repeated. In the meantime, the laboratory reported as follows:

Kahn test negative.

Hæmoglobin 70 per cent.

Red blood count, 3,790,000.

White blood count, 7,400

Differential white blood count

Neutrophiles, segmented, 60 per cent.

Neutrophiles, unsegmented, 3 per cent.

Eosinophiles, 8 per cent.

Monocytes, 5 per cent.

Lymphocytes, 24 per cent.

Sputum.—Essentially negative. *Urine*.—No significant change.

Stool—Mucus, uncinaria ova, and an unidentified ovum to which Miss THOMAS, the chief technician, called our attention, and regarding which we held a consultation. The ova were numerous, each low power field containing one or more of them. They were bile stained, and resembled very closely the ova of *Trichuris trichiura* save that they were slightly larger (approximately the size of the *Necator americanus* ovum), and that the ends of this unidentified egg lacked the small lemon-like projections which are so typical of the whipworm eggs. Instead, each end contained a small operculum. Miss THOMAS called our attention to the absolute similarity of this ovum to the picture in CRAIG and FAUST (1945), *Clinical Parasitology*, of *Capillaria hepatica* ova. This made the morphological identification absolutely certain. The following excerpt is taken from Stitt's *Tropical Medicine*—

"Among other Trichuridae is *Capillaria hepatica*, a very common parasite of the liver of rats and other rodents and the chimpanzee, and some monkeys, less commonly the dog. The eggs resemble those of *Trichuris*, but have an outer shell, a pitted surface, and measure 51 to 67 μ by 30 to 35 μ (Faust). They accumulate in the liver and form dry, yellow mottlings. The eggs remain in the liver and after the death of the host they may be freed by decomposition. Infection may occur by the ova being transmitted by flies to food, or by the eating of infected liver. Only one valid human case has been reported, from a British soldier in India, but some thirty cases of temporary or pseudo infection have been published, in which the ova were, presumably, eaten with livers of infected animals in Panama, French Guiana, Southern Rhodesia, and Russia (FAUST, 1931, VOGEL, 1932, SANDGROUND, 1933). FOSTER and JOHNSON (1939) refer to the probable origin of the cases in man in Panama. They found the white-lipped peccary, *Tayassu pecari spiradens*, the red spider monkey, *Ateles geoffroyi*, and the white-faced monkey, *Cebus capucinus imitator*, all infected. Some Panamanians eat all of these animals. On feeding boiled infected livers to other healthy monkeys, FOSTER found infection resulted."

After the second morphine injection was given, the patient was relieved and remained absolutely free from pain thereafter. For removal of the uncinaria, oil of chenopodium was administered on the day after they were reported by the laboratory. The chenopodium was given in accordance with our routine for women in this hospital, namely, three doses of 10 minims each, put up in gelatine capsules and administered at 2-hour intervals, the last capsule being followed between 2 and 3 hours later by 2 oz. of castor oil mixed with sodium bicarbonate and lime juice.*

Two subsequent stool examinations were reported by our laboratory as negative for *Capillaria hepatica* ova.

*See articles regarding chenopodium therapy by BISHOP and BROSIUS in the *AMA Journal*, published in 1917 and 1920.

NON-PIGMENTED MALARIA PARASITES IN THE BONE MARROW FROM A MIXED INFECTION OF *LEISHMANIA* AND *PLASMODIUM VIVAX*

BY

M YOELI, M D (former Lieut-Colonel, R A M C),
Department of Parasitology, The Hebrew University, Jerusalem

In July, 1945, a number of sternal puncture smears were obtained from a patient suffering from kala-azar in a civilian hospital in Athens * The smears were air dried, fixed in methyl alcohol and stained with Grubler giemsa in the usual way (one drop per 1 c c stained for 35 minutes)

On examination of the stained films a very heavy infection of *Leishmania*, both intracellular and free, was found Most of the parasites were in reticulum cells, but a few polymorphs were also found infected In the same smears a considerable number of malaria parasites were detected Their morphological features closely resembled those of *Plasmodium vivax*, with the striking exception that no pigment was seen in their cytoplasm, nor was pigment found in any phagocytic cells Most of the parasites were in an advanced stage of development, mature division forms and growing schizonts forming the majority The merozoites varied between 14 and 24 in number The full-grown schizonts filled the whole infected red cells In many the outline of the parasitized red cell was blurred, in others the outline of the host cell could be seen distinctly Apart from the absence of pigment, the parasites appeared to be normal and showed no degeneration The cytoplasm of the non-mature schizonts stained pale blue, whereas that of the mature parasites stained deep blue Only a few gametocytes were observed in the smears They showed no distinct pigment and occasionally one or two small vacuoles were seen in their cytoplasm No abnormalities were noted apart from the very striking one mentioned

* Dr A KHODUNIS, Director, Department of Clinical Pathology, The Red Cross Hospital, Athens, under whose care the patient was, submitted a report on this case to the clinical society of Athens I am indebted to him for the permission to use this case

In many fields leishmania and malaria parasites were seen side by side. In several examinations of the peripheral blood (thick drop method), before sternal puncture, no malaria parasites were found.

DISCUSSION

Mixed infections of kala-azar and malaria in the Mediterranean basin are rare.

Non-pigmented human malaria parasites were described by RAFFAËLE⁴ from smears of bone marrow obtained from patients with induced malaria. Their existence was correlated to the primary development phase of the human malaria parasites.

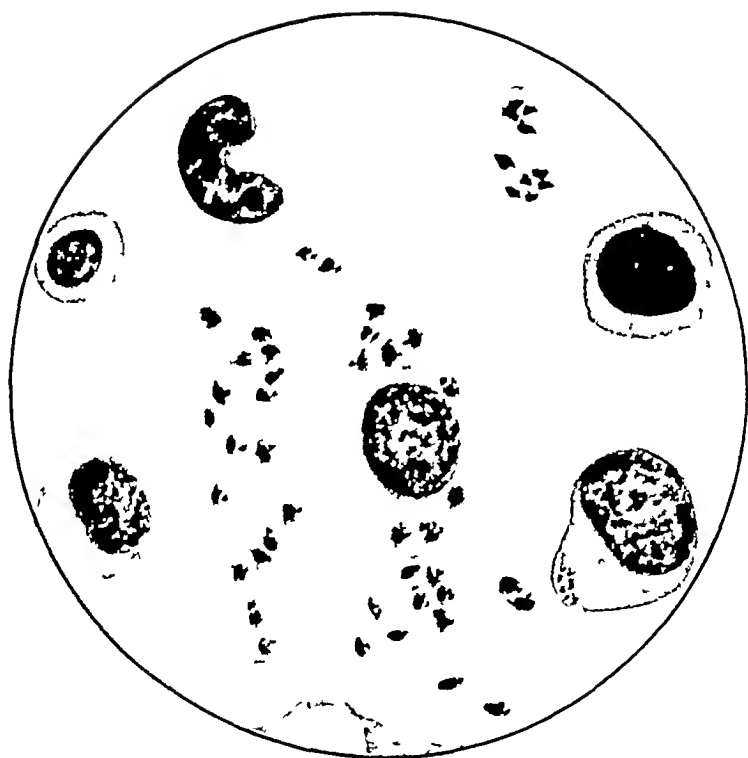
In the present case the development of non-pigmented forms, including gametocytes, occurred exclusively in the red cells, and we saw no indication of extra-erythrocytic development.

Whilst in Rome early in 1945, Professor RAFFAËLE very kindly showed the author the original smears on which his description was based, and the similarity of the parasites in his smears to some of the present forms is very striking.

The absence of malaria pigment in so many parasites can hardly be explained by mechanical factors during the preparation of the films. It is possible to conceive of changes not detectable by present staining reactions, which involve failure to produce malaria pigment. We could find no indication of the latter in the literature. Non-pigment producing strains of *P. vivax* have not been recorded. Another possibility presents itself i.e. that *P. vivax* after undergoing an extra-erythrocytic cycle, produces a generation of non-pigment producing parasites in red cells—here, too we have no direct evidence, but are induced to consider this possibility because of the similarity of some of the parasites in Professor RAFFAËLE's original preparation to those found in our slides.

Whatever the correct explanation of the present finding of non-pigmented parasites of *P. vivax* (including gametocytes and mature schizonts) may be, it is worth recording—particularly at a time when the problem of the development of the earlier stages of malaria parasites in man is under investigation.

RAFFAËLE, G. (1940) Ulteriori ricerche sulla fase monogonica dei plasmodi nell'uomo negli uccelli. *Riv. Malariol.* 1, 183



Camera lucida drawings of microscopic fields showing leishmania and a non-pigmented malaria parasite

INJURIES PRODUCED BY TROPICAL "WATER-BEETLES"

BY

K VIGORS EARLE, M D (CAMB), M D (LOND), M D (CUENCA)*

Medical Director, International Ecuadorean Petroleum Co, Guayaquil, Ecuador

The rainy season on the Ecuadorean littoral is characterized by plagues of various insects, one of which, the *cucaracha de agua* or giant water-beetle, has a bad reputation on account of its painful bite

The insects of this group, met with in Guayaquil (Provincia del Guayas, Ecuador), are

(1) *Lethocerus (Amorgius) camposi* Montandon (see illustrations), the largest form, which measures up to 85 mm (CAMPOS 1929)

(2) *Lethocerus (Lethocerus) annulipes* Herrich-Schaffe, similar to the previous one, but smaller in size (up to 60 mm)

(3) *Belostoma boops* Dufour, again similar to the previous one, but still smaller in size (up to 25 mm)

These Hemiptera belong to the family Belostomidae. Their metamorphosis is passed in ponds, especially those containing filthy water, and CAMPOS (1929) states that the larval forms may be found in the mud of dried-up ponds, cess-pools, sewers, ruts of waggons, marshes, etc., during the dry season. In the wet season (December to May), the insects invade the city in varying numbers, evidently attracted by the lights along the water-front of the Río Guayas. Some nights just a few are found whilst at other times many thousands congregate and are encountered at the foot of the electric-light standards, where they have fallen, stunned, after flying violently against the bulbs. This tendency to be attracted by bright lights has been observed in other members of the family.

The adults are predacious animals, feeding on all kinds of insects (usually aquatic), fish, salamanders (LEIDY 1847) and frogs—these latter are grasped with the powerful forelegs (PATTON and EVANS 1929) and the beak is driven into the lymph space (CAMPOS 1946). Even more remarkable is the fact that the insect may attack and kill birds—thus MATHESON (1932) describes an attack upon a good-sized woodpecker by *L. americanus*, in which the beak of the insect was inserted deep into the posterior part of the skull, after which blood and brain matter was sucked out.

* I have to acknowledge the kind assistance of Professor FRANCISCO CAMPOS, Section of Entomology, National Institute of Hygiene, Guayaquil, in the identification of these insects. The photographs are by Mr ABRAHAM MARTINEZ.

As will be noted in the illustrations, the insect has a quite ferocious appearance principally due to its large size. Figs. 1 and 2 depict anterior and posterior views respectively of *L. campestris* the specimen illustrated being captured in Guayaquil.

It may be readily imagined by analogy with the case of other powerful insects, that quite a number of fanciful attributes have grown up around this animal. Thus it is stated to leap on to unwary humans—which is untrue. It does not deliberately attack man and only inflicts a bite if handled—and even then only rarely. The insect which has been partially stunned by striking



FIG. 1—*Lethocerus (Ansergini) campestris* Montandon. Approximately natural size

against a light, is very sluggish in its movements and usually makes no attempt to bite, even when handled.

Although it has been alleged that the insects kill fish by inflicting poisonous punctures (FOLSON and WARDLE 1934), evidence as to the existence of actual poison glands is still somewhat obscure. The salivary glands of *Lethocerus* have been studied by LEIDY (1847) and LOCY (1834) there are two pairs—one pair elongated and extending posteriorly as far as the anterior extremity of the abdomen, the second pair about one-quarter the length of the first. The glands lie on either side of the oesophagus, on each side of which is a sigmoid swelling which might possibly be a poison reservoir. On the ventral aspect of the head, opening just above the base of the beak, is a pair of quite prominent glands, named by LOCY (1834) cephalic glands they are the homologues of

the maxillary glands in other Hemiptera Whether these produce venom or not is uncertain RILEY and JOHANSEN (1938) believe that it is the salivary glands themselves which produce poison

LESIONS IN MAN

As has been stated above, there is no deliberate attack upon human beings, but a painful puncture may be inflicted if the insect be handled "Back swimmers" (water-beetles which swim on their backs), belonging to the family

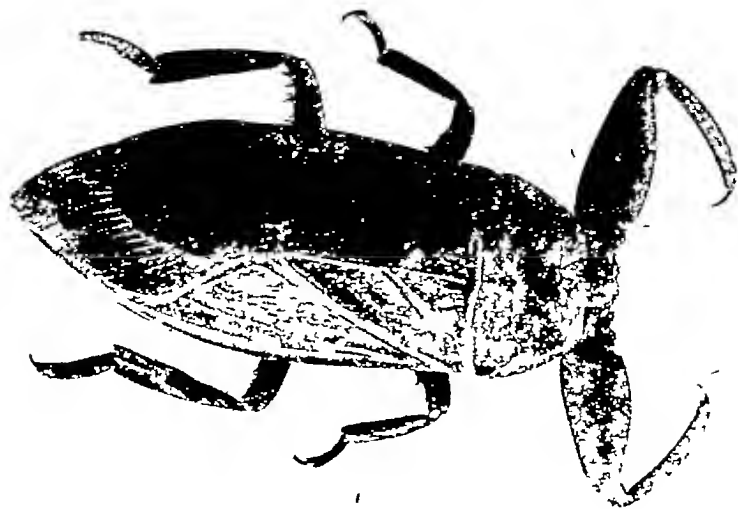


FIG 2—*Lethocerus (Amorgus) camposi* Montandon Approximately natural size

Notonectidae, are said by EWING (1928) to inflict a painful bite almost as severe as a bee-sting—but the bite of the group under consideration is very much worse, it is followed by temporary blanching around the puncture-wound, and this is followed by swelling, redness and throbbing Normally the lesion disappears within 24 hours, but in some cases may go on to cellulitis and supuration, with limited gangrene, in such cases the regional lymphatics usually show an acute adenitis In Ecuador there is a widely held belief that owing to the habitat of the insect (sewers, collections of filthy water), its bite is very much more severe and prone to sepsis than equally vicious injuries inflicted by insects of cleaner surroundings Some cases of tetanus have been said to

have been consequent to the bite, although I have been unable to find any authenticated case.

EWING (1928) in a study of the giant water bug *Belostomatidae* Say makes the following observations: at 9.30 a.m. the insect was allowed to bite the back of the index finger. The beak was left inserted for a few seconds only but almost immediately there was a burning sensation in the affected region. This was soon followed by swelling, with redness appearing around the puncture site. The pain continued, but in diminishing amount, throughout the forenoon. At noon the area of redness had reduced considerably and by 1.30 p.m. a small red spot was all that was visible at the point of puncture. The same author observes that when this insect bites it emits a milky fluid at the end of the beak and the beak adhering to the skin after biting, the skin is pulled up as the beak is withdrawn. RILEY and JOHANNSEN (1933) state that when *L. americanus* bites the hand severe shooting pains proceed up the arm and are present for several days. These observers recommend the use of ammonia or of menthol ointment as treatment.

TREATMENT

Immediate first aid consists in the application of ammonia to the puncture site, as already indicated above. In the case of septic complications, the use of sulphur drugs or penicillin is indicated. As an additional precaution, prophylactic antitetanic serum should be administered.

SUMMARY

- (1) An account of giant water beetles which occur in Ecuador along the banks of the Guayas River is given.
- (2) Effects of the bites of these insects, together with the observations of other authors, are described.
- (3) Treatment of the bites is outlined.

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[The previous number of these Transactions, Vol 42, No 1,
was published on July 27th, 1948]

TRANSACTIONS OF THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE

TO INTENDING CONTRIBUTORS OF PAPERS

The following letter dated June, 1933, is republished as despite the fact that the Editorial Notice was rewritten as suggested, the same faults are still to be found in papers being submitted today. It should be added that the cost of producing a page of the TRANSACTIONS is now more than double the figure given.

"SIR,

It has become evident to us that the Editorial Notice appearing in each issue of the TRANSACTIONS and addressed to authors submitting papers for publication is frequently disregarded by them, at least in so far as the desirability of writing concisely is concerned. Many of the papers received by us for consideration are extravagantly long and in consequence we have to advise their curtailment. Further, some authors are unduly lavish in their employment of tables. Besides taking up space, tables are very costly to reproduce and some proportion of those sent in for publication add little or nothing to the matter they are intended to illustrate. It is impossible to enlarge the TRANSACTIONS further, for each page costs over £1 to produce. Therefore, in order to make room for the increasing numbers of valuable papers submitted for publication we consider that the Editorial Notice should be rewritten in such a way as to impress on authors the importance of avoiding any unnecessary expansion of their subject matter.

Yours faithfully,

WILLIAM FLETCHER	} Members of the Editorial Panel "
W P MAC ARTHUR	
WARRINGTON YORK	

to use Manson House as a centre where they could meet, as at a club.

The President replied that the Council will consider Dr NAPIER's suggestions and see how they can be met.

The adoption of the Report was proposed by Dr NAPIER, duly seconded, and the resolution was carried.

REPORT OF THE HON. TREASURER FOR THE YEAR ENDED 31st MARCH 1948

The Hon. Treasurer Dr J. C. BROOM, presented his Report, with the accounts and balance sheet prepared by the auditors, Messrs. W. B. KERN & Co., and approved by the Audit Committee.

Dr BROOM pointed out the satisfactory financial position of the Society and that the number of Fellows continues to increase, being 103 above last year's figure. The balance of income over expenditure was £88, but it is expected that the income received from resident tenants (now £1,025) will be considerably increased next year.

The adoption of the Treasurer's Report was proposed by Dr NAPIER. The resolution was seconded and carried.

ELECTION OF AUDIT COMMITTEE.

Dr BAGSTER WILSON proposed that Drs. W. E. COOKE, C. A. HOWE and P. C. C. GARNHAM be appointed to the Audit Committee. This proposal was seconded by Dr F. HAWKING, and carried unanimously.

PRESENTATION OF CHALMERS MEDALS

The President: We now come to the business of the evening, the presentation of the Chalmers Medals. First, for 1941 to Dr JOHN WILLIAM FIELD whom we salute as one who has earned our esteem not only for his work in tropical medicine, but also for his staunch personal qualities in time of war. Dr FIELD first sprang into fame as a medical officer in the Malayan Medical Service from 1925-31 subsequently as Senior Medical Research Officer at the Institute of Medical Research in Kuala Lumpur. In February 1942, he was interned by the Japanese. In Singapore, Dr FIELD has accomplished a great amount of research work on mite typhus, on vitamin A deficiency and the preparation of vaccine lymph in Malaya. His chief contribution has been on the chemoprophylaxis of malaria, the morphology of the parasites, and especially his thick film staining technique which has been put to such excellent use in the mass diagnosis of malaria in field surveys in hyperendemic areas. Field's stain now holds a world-wide reputation, having the advantage of saving much valuable time to the laboratory worker. His publications are characterized by high technical ability, marked originality and a rare capacity for analysing his results. This was seen especially in his work on atebirin chemoprophylaxis, in showing its high suppressive efficiency in doses of 0.2 grammes twice weekly in an Indian labour force. This more than any other published work at that time led to the official adoption of this method of malaria prevention in the armed forces during the recent war.

In view of the fact that Dr FIELD has returned to Malaya, his son, Mr MERVIN FIELD, will receive the medal on his behalf.

The Chalmers Medal for 1943 is awarded to Dr ROBERT KIRK Dr KIRK has earned world-wide fame in the Sudan, thereby rendering inestimable services to that country, and in so doing has worthily trod in the footsteps of his predecessors, Sir ROBERT ARCHIBALD and Sir ANDREW BALFOUR During 14 years' service in the torrid Sudan, he has with great energy continued to turn out original work of great value He has studied the sandflies of Ethiopia, and the various and puzzling forms of leishmaniasis in man, and more than anyone else has demonstrated the relationship of their clinical forms to one another His evaluation of the different drugs employed in treatment is especially valuable, and he was the first to point out the bizarre toxic manifestations of stilbamidine In 1941, Dr KIRK was engaged in investigating the surprising and unexpected outbreak of yellow fever in the Nuba Mountains By the immediate steps undertaken to prevent its spread, he averted a situation that might have seriously retarded the outcome of the war in Abyssinia Dr KIRK is essentially a field worker, who is seen at his best with difficult field conditions He has deserved well of his country and of tropical medicine, so that it is with great pleasure that I hand him the medal he has so richly earned Brigadier BOYD will receive the medal on Dr KIRK's behalf

The Chalmers Medal for 1945 has been awarded to Dr E M LOURIE, of the Liverpool School of Tropical Medicine For 17 years Dr LOURIE has been engaged upon intricate research work, and he has been associated with several famous figures in Tropical Medicine—with SAUL ADLER, in Jerusalem, with TALIAFERRO, in Chicago, and with WARRINGTON YORKE At present he is regarded by many as the natural successor of this latter great worker Dr LOURIE is one of the pioneer research workers on the action of drugs in bird malaria, and his technique has since been adopted and perfected by others on both sides of the Atlantic Together with WARRINGTON YORKE, he was active in the elaboration of the diamidines and their application to trypanosomiasis and leishmaniasis From 1934 to 1937 he held a Beit Scholarship, and he is now Director of the Warrington Yorke Department of Chemotherapy His more recent researches have been directed to the action of penicillin in spirochaetal diseases, and he is now generally acknowledged as the pioneer of the ambulatory treatment of syphilis by massive doses at appropriately spaced intervals During the recent war, Dr LOURIE was in Sierra Leone investigating a serious epidemic of sleeping sickness, and there he initiated field trials of drugs of the diamidine group Dr LOURIE, then, has earned outstanding laurels in a particularly difficult field of work, and it is therefore with a sense of profound admiration that I hand him the Chalmers Medal

Dr E M Lourie Mr PRESIDENT, I am afraid you did not exhaust the list of people to whom I owe whatever I may have learnt or achieved in the field of tropical medicine It was, in fact, yourself, together with Dr CARMICHAEL LOW, who first introduced me to the mysteries of tropical medicine,

CLARK and GRAY (1935) recorded a similar case. DAY (1937), MAUSNER (1938), SILVEIRA (1944) and KENAWY (1947) published articles on pulmonary schistosomiasis.

INCIDENCE.

In Egypt, 60 to 70 per cent. of the inhabitants are infected with schistosomes at least 33 per cent. of these have pulmonary schistosomiasis.

The disease affects males more often than females and is most common in children and young adults, the highest age incidence being between 10 and 30 years. It is a disease of the agricultural workers.

PATHOLOGY

Pulmonary schistosomiasis is the result of the deposition of schistosome ova and worms in the lungs. These reach the lungs as emboli from the veins of the urinary and intestinal tracts. From the veins of the urinary tract they reach the lungs by way of the internal iliac veins and from the veins of the intestinal tract by means of the porto-caval anastomoses.

S. haematobium ova are more often found in the lungs than *S. mansoni* ova, but the latter produce vascular lesions more frequently than the former.

The ova, reaching the lungs as emboli, obstruct the small arterioles, pass through their walls and lie immediately outside them. The tissue reaction round the ovum results in the formation of the bilharzial tubercle. This consists at first of the ovum surrounded by histiocytes and eosinophil leucocytes. Later lymphocytes and one or more giant cells appear. Fibroblasts invade the tubercle and a nodular scar is formed.

The ova produce two types of lesions, parenchymatous and arterial.

Parenchymatous lesions lie in relation to the bronchioles and alveoli. They are scanty in number and produce little alteration in the finer structure of the lungs.

Arterial lesions, though less frequent, are more serious. They are the result of the necrosis of the walls of the arterioles produced by the passage of the ova through them. Necrosis is followed by healing with thickening and narrowing or occlusion of the lumen of the vessels. Canalization of the obstructed vessels may occur with the formation of angiomatoids.

The arterial lesions may be confined to few vessels or be widespread. In the latter case dilatation and thickening of the arteries in the lungs and of the pulmonary artery and its primary branches result. The right ventricle dilates and hypertrophies and finally fails.

To the naked eye at a comparatively early stage, the bilharzial tubercles appear as milary nodules on the cut surface of the lungs. These are about 0.5 to 1 mm. in diameter greyish white in colour firm in consistency and are arranged in clusters or lines round the thickened arteries. At a later stage these nodules disappear and only thickened dilated vessels are seen.

The pulmonary artery and its primary branches are dilated and may reach

aneurysmal size Their walls may show atheromatous changes and their lumina may contain antemortem thrombi The right ventricle is dilated and hypertrophied and its wall may be 1 cm or more in diameter The right auricle may also be dilated

Schistosome worms may be present in the blood vessels of the lungs While living they are harmless, but when dead they produce a necrotic and focal pneumonia which appears as opaque white areas of consolidation 0.1 to 0.5 cm in diameter

CLINICAL PICTURE

Clinically, two forms of pulmonary schistosomiasis can be recognized bronchopulmonary and cardiovascular

In the bronchopulmonary form the pathological lesions are in the bronchioles and alveoli Clinically, these manifest themselves in the form of bronchial asthma, chronic bronchitis, bronchiectasis, pulmonary emphysema and pulmonary fibrosis

In the cardiovascular form the pathological lesions are primarily in the pulmonary arterioles and, secondarily, in the pulmonary artery and its branches, and in the right ventricle

The clinical picture of this form is similar to that of primary pulmonary endarteritis or Ayerza's disease However, cyanosis, which is the most striking feature of Ayerza's disease, is absent in pulmonary schistosomiasis, and appears only when the right ventricle has failed

The cause of the cyanosis in Ayerza's disease is not clear FISHBERG (1940) states that marked cyanosis in Ayerza's disease, without failure of the right ventricle, "indicates the presence of changes in either the capillaries or the pulmonary parenchyma interfering with gas exchange" BEDFORD (1946) is of opinion that "the classical clinical picture of so-called Ayerza's disease is really one of chronic lung disease and heart failure combined"

The electrocardiogram shows right heart strain (right ventricular preponderance) Arrhythmias, other than extra-systoles, have not been observed.

Radiologically, at an early stage some of the small branches of the pulmonary artery in the lungs, usually the basal, show nodules about 1 mm in diameter along their course At a more advanced stage the nodular arteries are more numerous, the hilar shadows are increased in size and the subaortic notch is obliterated At a still more advanced stage the hilar shadows are very much increased in size and the pulmonary artery and conus are very much enlarged, may reach aneurysmal size and mask the shadow of the aorta and its knob The heart has the configuration of *cor pulmonale*

DIAGNOSIS

The only certain means of diagnosing pulmonary schistosomiasis is the presence of schistosome ova in the sputum However, they are very rarely found in the sputum

Establishment of the presence of schistosomiasis is essential. This consists in the demonstration of ova in urine or faeces, sigmoidoscopic evidence of schistosomiasis, rectal and liver biopsy for ova, and the specific tests of schistosomiasis. Eosinophilia is more marked in pulmonary than in other forms of chronic schistosomiasis.

Examination of the lungs clinically is of little help, as there are usually no signs or only few crepitations and rhonci over their bases.

Examination of the heart clinically and radiologically for signs of *cor pulmonale* is important. Other causes of *cor pulmonale* have to be excluded, specially mitral stenosis and atrial septal defect. Mitral stenosis can be excluded by its characteristic cardiac signs, and dilatation of the left auricle observed radiologically. Atrial septal defect is differentiated by early dilatation of the right auricle, hilar pulsation and absence of nodular arteries in the lungs. At a late stage differentiation may be possible only by auncular catheterization.

Radiologically military tuberculosis and sarcosis have to be differentiated from pulmonary schistosomiasis.

In military tuberculosis the lesions in the lungs are widespread, and there is no dilatation of the pulmonary artery and conus. In sarcosis the lesions are peribronchial and there may be extensive fibrosis in the lungs.

PROGNOSIS.

It has been mentioned previously that 60 to 70 per cent. of the inhabitants of Egypt are infected with schistosomiasis and that one-third of these have pulmonary schistosomiasis. Postmortem records shows that 2 per cent. of subjects of schistosomiasis die from right ventricular failure as the result of bilharzial pulmonary arterial disease.

Clinically bilharzial pulmonary arterial disease is a much less common cause of right ventricular failure than rheumatic heart disease, hypertension and other chronic pulmonary diseases. Right ventricular failure is late in appearance as its cause is mechanical rather than myocardial.

As a cause of chronic pulmonary disease, such as chronic bronchitis and bronchiectasis, it is difficult to give an accurate idea of its importance as it is often difficult to establish the bilharzial origin of these diseases.

TREATMENT

Early treatment with antimony compounds is important. In advanced cases showing enlargement of the right ventricle they must be used with caution as they are toxic to the myocardium. They are definitely contra indicated when the right ventricle has failed.

Heart failure is treated on ordinary lines by digitalis, venesection and mercurials. Recently McMICHAEL (1949) has expressed the opinion that digitalis and venesection, in heart failure of pulmonary origin, are unlikely to help and may even be dangerous. This view however is not shared by other authorities.

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DISCUSSION

The President It is really with great pleasure that I commend this very excellent clinico-pathological paper of Professor ERFAN'S. In his clinical demonstration he has shown a mastery not only of the physiology of the lung, but also of the very difficult interpretation of the radiological pictures, in which one can see the thickened arteries and the pulmonary sclerosis very well indeed. Many of us would like to make remarks upon this intricate subject which he has presented in a novel way.

Sir Henry Tidy It has been a great pleasure to listen to my friend, Professor ERFAN. The study of bilharziasis reflects great credit on the Medical School of Cairo, and in the last 20 years important advances have been made. Twenty years ago Professor DAY had not yet quite persuaded the Medical Faculty that the so-called Egyptian Banti's disease was bilharziasis. That discovery was an enormous advance. Yet the School has gone further. Dr HASHEM, in the Children's Hospital, is now differentiating cirrhosis of the liver of bilharziasis type in children from cirrhosis of the liver due to protein deficiency. The recognition of pulmonary bilharziasis is almost entirely the work of the Cairo Medical School, a number of whose members have been concerned in the research. The credit for the first step must be given to Professor SOROUR, the pathologist who accurately described the changes in the lung in 1928. In 1932, Dr AZMY and Dr EFFAT described the first clinical case. I remember, shortly after the case was published in 1932, discussing it with Dr AZMY. He said, "We have in Egypt an excess of rather curious lung changes," and he suggested that pulmonary bilharziasis was a frequent cause. Both in Professor ERFAN'S wards and my own wards I have seen many cases that one could have accepted as pulmonary bilharziasis, but, as he tells us, the diagnosis is sometimes very difficult. I would like to say a few words about Ayerza's disease, for this name is sometimes used in Egypt for these cases. The textbooks all state that Ayerza's disease is characterized by very

deep cyanosis due to sclerosis of the pulmonary artery which is believed to be syphilitic in origin. The ascription of Ayerza's disease to syphilis is curious. Sir LEONARD ROGERS described a few cases in India, and added that some of these cases occurred in young people and therefore could not be degenerative, and were probably syphilitic. From that casual remark, Ayerza's disease has been ascribed to syphilis, which is certainly incorrect. The best description of the pathological lesions in Ayerza's disease is given by CHERRY in the *American Journal of Medical Science*. It may be noted that the subject had been a miner. I can see no essential difference between his illustrations of the arterial changes and those which can be reproduced from cases of pulmonary bilharziasis in Egypt, but it is difficult to judge from pictures. There is a further point with regard to the explanation of the cyanosis. Many of these cases in South America with advanced cyanosis come from the uplands, from the Andes, where people live at an altitude of 14 000 to 16 000 feet, and are engaged in mining. It is also reported that when these patients were brought down to the level, the cyanosis greatly diminished. Thus, admittedly is in the last stage with cardiac failure. These cases were not fully described, and it is not clear when or why the cyanosis develops. Quite possibly it is due partly to local causes developing in high altitudes and partly to pulmonary conditions arising in the mining industry and not directly to the pulmonary arteritis. The possible factor of pulmonary bilharziasis as the cause of pulmonary arteritis in the South American cases also has not been considered. In Egypt pulmonary arteritis does not produce cyanosis. Egypt is a flat agricultural country with no mines. I believe that the name Ayerza's disease should be restricted to this particular syndrome in South America, and not applied, as it sometimes is, to every case of pulmonary arteritis dying in cardiac failure.

With regard to the intensive course of tartar emetic in the treatment of bilharziasis introduced by BLAIR and ALVES, I understand that some workers in South Africa have had bad reactions, but less severe than those in Egypt. The subjects in South Africa were generally otherwise healthy whereas in Egypt there is nearly always *Ancylostoma* and one or two other infections. Those undertaking this method should be very careful.

Professor Day. I was much interested to hear Professor EAPAN speaking on a subject to which he has devoted so much attention. There were one or two points which I found extremely interesting and very intriguing. I remember that years ago Professor LOOSE—who was so devoted to worms that he used to swallow ova and grow some himself—remarked that if ever a cure was found for bilharziasis the effects might be worse than the disease. He feared that the death of the worms might cause serious thrombosis, especially in the portal vein.

When last in Egypt I was shown a series of liver sections which had been prepared by Dr RAMSES GIRCUI, of Tanta, from cases of hepatic bilharziasis. Many of them showed an extraordinary picture similar to the condition illustrated in some recent papers on filariasis. The amount of necrosis and cellular

infiltration around dead bilharzia worms in the liver is something staggering I can imagine that when worms get to the lung and die there they will provoke a good deal of local reaction

There remains the question Why does mansonii infestation appear to be more commonly associated with severe arterial changes in the lung than is found in haematobium cases? I cannot help feeling that we do not know the proportion of cases in which lung changes are due simply to embolism of ova compared with those where bilharzia worms are present and deposit ova in the pulmonary arterioles, a condition which may lead to widespread arteritis I do not wonder that Professor ERFAN regards himself as a professor of clinical rather than of tropical medicine when we realize the diagnostic difficulties and investigations required for the distinction between pulmonary bilharziasis and other conditions which affect the lungs and heart

There is just one point of differential diagnosis which Professor ERFAN may have noted We hear that in the severe cases there is a dilatation of the pulmonary arteries This may involve the conus and the pulmonary valves may become incompetent Is there a pulsating shadow to be seen on the screen in the hilar region around the heart? I do not know whether this has been observed (it would not appear in radiographs), but think it might be a valuable sign in cases of vascular dilatation

Dr C C Chesterman I have been particularly interested in this excellent lecture because I feel that a clinical observation which I put on record 25 years ago has been justified tonight

I refer to pulmonary symptoms in infestation by *S. intercalatum*, that other bilharzial worm found in the Belgian Congo

It is still a question as to whether it is a new species or not, but clinically it is an entity and its localization always intestinal Dysenteric symptoms are constant but few other complications develop People get a certain amount of immunity in later years so that it was not found possible to reinfest them

I noticed in young people, however, that they frequently got attacks of pleuritic pain and pneumonia along with the initial dysentery Diligent search for ova in the sputum was made On one occasion a patient was found sitting on the floor in a paroxysm of coughing, and beside him a specimen of blood stained mucus, which, on examination, was full of the characteristic ova Unfortunately, investigation proved that it had come from the other end of the alimentary canal

Although I can adduce no pathological confirmation, I believe that pulmonary symptoms do occur in this disease

I agree with the possible dangers of too energetic treatment with tartar emetic Among other things it is apt to light up pulmonary tuberculosis

Mr Alves Professor ERFAN has emphasized the paucity of reports of ova in the sputum, and I would like to mention that BLAIR and I have

recorded—rather *eter aba* I am afraid—a European case sent to us for skin-test. He had bronchiectasis, and both urine and stool were negative for ova, although his cercarial-antigen skin test was strongly positive. He produced a pint of sputum for us and, after concentration with KOH and centrifugation of the digest, I found *one* terminal-spined egg. Difficulty in finding ova in sputa is therefore not surprising.

With regard to Professor ERIAN's strictures, I would suggest that not only may there possibly be a racial difference between Bantu and Egyptian in their ability to tolerate antimony as I have already mentioned to him, but also I would stress that the schistosomiasis of Egypt is almost a clinical entity in its severity as compared to the rest of Africa. I wish some work could be done, possibly in my own country where ample material exists, and in Egypt, on total worm counts in cases coming to autopsy. We might then find a definite reason for this disparity.

With regard to his beautiful X rays of pulmonary schistosomiasis, I do not think the diagnosis of pulmonary schistosomiasis is very often made in Southern Rhodesia, if at all, but I would emphasize that silicosis and tuberculosis which would, I think, give very similar appearances, complicate the picture there.

Professor ERIAN. Dr ALVES, I am rather surprised that pulmonary schistosomiasis has not been diagnosed in South Africa. I understand from TURNER'S examination of postmortem cases that pulmonary schistosomiasis is quite common in South Africa.

Mr ALVES. I meant my remarks to apply only to X ray diagnosis. My colleagues in Southern Rhodesia have completed a very full and comprehensive study of the distribution of ova throughout the body and eggs in the lungs are a commonplace finding.

Professor ERIAN. The short intensive antimony treatment of ALVES and BLAIR of schistosomiasis has not been successful in Egypt as it often produces severe, sometimes serious, general reactions. This may be due to the disease being severer in Egypt than in South Africa, and to anaemia and nutritional deficiencies being common in the former.

I agree with Professor DAY a statement that hilar pulsations are marked in interatrial septal defect, and this may be helpful in the differential diagnosis. Hilar pulsations are not marked in bilateral pulmonary arterial disease as the walls of the vessels are rigid from atheromatous changes.

The President. I think we have had a most interesting evening from every point of view. I hope, Sir, you will take back to Egypt our great appreciation of the manner in which you have delivered your lecture tonight.

COMMUNICATIONS

OBSERVATIONS ON THE DEVELOPMENT OF RESISTANCE TO VIVAX MALARIA

BY

C R BICKERTON BLACKBURN, M D, M R C P, M R A C P,*

*Joseph Thornton Tweddle Research Scholar for the Royal Australasian College of
Physicians †*

TABLE OF CONTENTS

INTRODUCTION	PAGE
PART I	
EXPERIMENTS DESIGNED SPECIFICALLY TO SHOW THE DEVELOPMENT OF TOLERANCE AND IMMUNITY IN VOLUNTEERS WITH MOSQUITO TRANSMITTED VIVAX MALARIA	120
PART II	
AN EXPERIMENT DESIGNED SPECIFICALLY TO SHOW THAT THE DEVELOPMENT OF TOLERANCE PRECEDED THE DEVELOPMENT OF IMMUNITY AND THAT IMMUNITY WAS STRAIN SPECIFIC	145
PART III	
THE DEVELOPMENT OF TOLERANCE IN GROUPS OF VOLUNTEERS WHO WERE USED IN EXPERIMENTS DESIGNED TO DETERMINE THE CHEMOTHERAPEUTIC ACTIVITY OF VARIOUS DRUGS	151
DISCUSSION	
CONCLUSIONS	

INTRODUCTION

Resistance to vivax malaria may be manifested in one or both of two ways

- 1 There may be less reaction by the host to a given bulk of infection—to erance
- 2 There may be an increased ability of the host to limit the bulk of infection developed—immunity

* I wish to express my indebtedness to Dr N HAMILTON FAIRLEY, not only for his stimulating and helpful criticism, but also for providing the opportunity to carry out these studies. It is a pleasure to acknowledge the help of the members of the L H Q Medical Research Unit, Cairns, and especially of Dr M. J MACKERRAS, T S GREGORY, D VET SCI, and Dr R. H BLACK

† The experimental work was done in the L H Q Medical Research Unit, Cairns

The difference is important for a patient with malaria is concerned with the abolition of his reaction rather than in the absolute clearance of malaria parasites from his organs. Furthermore, the two forms of resistance appear to be distinct and do not develop at the same time.

The development of tolerance before the development of immunity to *Plasmodium vivax* in patients with induced malaria has been described by several authors (YORKE and MACFIE, 1924; BOYD and KITCHEN 1938; BOYD, 1938), and these patients were usually being treated for general paralysis. SINTON (1939) described similar phenomena in monkeys. JAMES (1931) and BOYD STRATMAN THOMAS and KITCHEN (1936) showed that early therapy of a malaria attack delayed the appearance of tolerance and immunity and that the host had to experience considerable reaction to his infection before he became tolerant or immune. The studies of JAMES (1931) and of BOYD and STRATMAN THOMAS (1933) revealed that immunity was strain specific but that some tolerance might be developed to heterologous strains of *P. vivax*. SINTON and HARBHAGWAN (1934) working with *P. knowlesi* infections in *Simulium theileri* and *S. pratti* showed that multiple superinfections with heterologous strains resulted in greatly enhanced tolerance and immunity but BOYD (1938) found that simultaneous infections with *P. vivax* resulted in less absolute tolerance and immunity to either strain than was obtained using either strain alone. BOYD and KITCHEN (1936) reported that tolerance and immunity were developed following either sporozoite or trophozoite induced vivax malaria and that this protection was evident against reinfection by both types of induced malaria.

An enormous number of studies of tolerance and immunity to malaria in populations in endemic areas have been reported, but no attempt has been made to review this literature in this report. These studies have been concerned, in the main, with the differentiation between "malaria disease and malaria infection" (SINTON 1945). The spleen and parasite rates have been used to determine the general state of the population, but interpretation of the findings has been difficult. CHRISTOPHERS (1911) gave a table showing very high spleen rates exactly paralleling lower parasite rates following the 1908 epidemic in Amritsar while SWELLINGERHEL (1939) cited a remote village in Dutch Guiana where he found high spleen rates, with higher parasite rates, in the absence of past or present clinical malaria. As HACKETT (1937) stated, the interpretation of malaria indices is not an exact science, but depends largely on experience and imagination. SINTON (1939) working with human malaria, described the maintenance of a high degree of tolerance by periodic stimulation by reinfection. This tolerance was usually associated with splenomegaly. BOYD (1941) regarded a persistently enlarged spleen as indicating in man, either continued liability to recurrences or continued reinfection with heterologous strains or species.

In spontaneously subsiding malaria, parasites may persist in the peripheral circulation long after the fever has subsided (YORKE and MACFIE, 1924) and,

with some strains, mosquitoes have been infected by gametocytes that were not found by microscopic examination of the blood (SWELLENGREBEL and DE BUCK, 1938) BOYD and KITCHEN (1937) also reported the infection of mosquitoes by densities of gametocytes less than 10 per c mm in the peripheral blood "Healthy carriers" have been described, though they seem to be common only in endemic areas in native populations

This report is concerned with the development of tolerance and immunity in volunteers who were healthy prior to experimental infection with *P vivax*, they had not previously been exposed to malaria

The degree of resistance of volunteers with vivax malaria was assessed by studying certain clinical and parasitological phenomena occurring during the course of the infection The clinical phenomena studied were the subjective and objective reactions, including the oral temperature, and the evidence of change in size of the liver and spleen in subjects with active malaria These were related to the trophozoite densities in the peripheral blood

Oral temperatures were recorded at least each morning and each evening in subjects without active malaria, each 4 hours in subjects with active malaria, and each 2 hours in subjects with overt malaria

Each man's "fitness," based on his usefulness to a combatant army unit, was assessed and recorded at least each morning either by the author or R H BLACK, M D The following categories of fitness were recognized —

- (a) Fit for front line duty no symptoms
- (b) Could continue on duty some minor symptoms but insufficient to cause the man to leave his duty
- (c) Fit for light duties minor symptoms of such a degree that a unit medical officer would order light duties
- (d) No duties for that day some major symptoms present
- (e) Evacuation from unit ordered major symptoms of such a degree to necessitate evacuation from the unit

The assessment of the man as an individual was found to be both more reliable and far more useful than an analysis of specific symptoms such as headache, malaise, vomiting or rigors The five categories described above were reduced to three to facilitate comparisons in certain parts of this report

- (a) Perfectly fit no symptoms
- (b-c) Light duties for some men minor symptoms only
- (d-e) Of no use to the unit major symptoms

If a man was recorded as being, say, (b) in the morning and (e) in the afternoon of a given day, it was arbitrarily decided to call his degree of fitness $b-e$ ($\frac{1}{2}e$, $\frac{1}{2}b$ $\frac{1}{2}e$) This was necessary to allow the calculation of averages for groups of subjects and their graphic representation

Trophozoite densities were recorded each day and these figures were treated as geometric series throughout. Curves were fitted to each trophozoite wave and suitable comparisons made with others from time to time. The logarithms of the mean parasite densities were converted into natural numbers before being expressed.

The phase of sporozoite induced malaria occurring between the inoculation of sporozoites and the liberation of trophozoites into the circulation will be referred to as the e.e. phase (exo-erythrocytic phase). This term will also be applied to whatever forms of parasite persist in relapsing vivax malaria after the peripheral blood has been cleared of trophozoites and gametocytes.

In this report, as with all reports from the Cairns Unit the day of infection is referred to as day 0 the next day being day + 1 etc. This applies to both sporozoite and trophozoite induced malaria.

The technical methods used in this study are those referred to in the earlier reports from the Cairns unit (FAIRLEY *et al.* 1945 1946a, 1946b 1947).

PART I

EXPERIMENTS DESIGNED SPECIFICALLY TO SHOW THE DEVELOPMENT OF TOLERANCE AND IMMUNITY IN VOLUNTEERS WITH MOSQUITO TRANSMITTED MALARIA.

Experiment 1 The development of tolerance in a volunteer with sporozoite induced vivax malaria who received quinine suppression (Volunteer 016000), (Chart 1.)

A 21 year-old man, who had not previously been exposed to malaria, received 0.7 grammes of quinine sulphate (as mixture) daily from day - 2 to + 63. His plasma was frequently collected immediately prior to the administration of the daily dose of quinine and contained a mean (minimum) concentration of 1.03 mg. of quinine (as base) per litre and the mean maximum concentration ($2\frac{1}{2}$ to 3 hours after the daily dose) was 5-60 mg. per litre.

He was bitten by ten *Anopheles punctulatus punctulatus* infected with viable sporozoites of *P. vivax* (C strain from volunteer C Chart 12) on day 0. The batch of mosquitoes was 83 per cent. infected, the gland infections were heavy and the sporozoites had been in the salivary glands for 5 days.

Trophozoites were first demonstrated in thick blood films collected on day + 13 and the densities increased to reach 7,300 per c.mm. on day + 28. Between day + 27 and day + 63 when treatment began, the densities varied between 800 per c.mm. and 10,500 per c.mm. (Chart 1.)

His oral temperature rose to 102° F on day + 13 and there were quotidian rises on the next 2 days, but from day + 16 to + 23 there were tertian rises in temperature. From day + 24 to + 63 there were quotidian rises in temperature to 100 to 106° F.

His spleen was first palpable below the left costal margin on day + 19 and reached a maximum of four finger-breadths below the costal margin on day + 50. His liver was first definitely palpable on day + 14.

Symptoms were first remarked on the evening of day + 13 and remained minor to day + 19 when he was first classified (d). Symptoms were tertian in severity till day + 24 corresponding with the rises in oral temperature but subsequently the daily record of "fitness" changed from () or (d-s) to (e) or (b-e) in spite of the daily rise in temperature to over 100° F. True major paroxysms (or agues) were recorded on days + 23 + 26, + 28 + 31 to + 43 + 45 to + 46 and + 48 to + 63; on four of the intervening

days minor paroxysms occurred, making a total of 39 paroxysms in 43 days. From day + 42 to + 60, this man spent his mornings fishing in a stream some 1 to 1½ miles from the unit, returning to have his paroxysm at 3 p.m. each afternoon. He spent his evenings playing cards or billiards.

The red blood cell count and haemoglobin concentration diminished from 4.68 million per c.mm. and 15.0 grammes per 100 ml. to 3.54 million per c.mm. and 9.5 grammes per 100 ml. respectively. His weight decreased from 159 lb. on day + 3 to 144 lb. on day + 66.

Standard Q.A.P.* treatment began on day + 65 and concluded on day + 77, after which no further drugs were administered.

He remained perfectly well and parasites were not demonstrated in thick blood films collected between the cessation of treatment and day + 91, when trophozoites were again seen. Densities increased in the peripheral blood to reach a maximum of 21,100 per c.mm. on day + 102 and remained between 260 per c.mm. and 8,200 per c.mm. till standard Q.A.P. was again administered on day + 129.

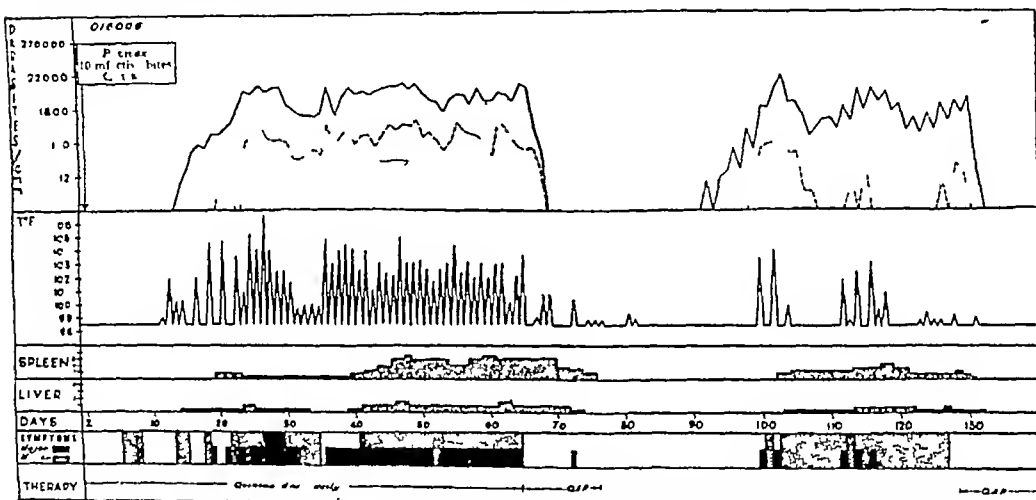


CHART 1—The development of tolerance in a volunteer with sporozoite induced vivax malaria who received quinine suppression (Volunteer 016006, Experiment 1)

His oral temperature did not reach or exceed 99°F till day + 100, when the parasite density increased from 1,740 per c.mm. to 9,000 per c.mm. His temperature record showed tertian rises from day + 100 to + 104 and between day + 112 and + 118, subsequent to which no temperature of 100°F or over was recorded. Symptoms were minor except for parts of days + 100, + 102, + 112, + 114 and + 116, when he was graded (a-d), (a-e), (b-e), (b-e) and (b-e) respectively. His spleen reached a maximum size of three finger-breadths below the costal margin on day + 118. His weight remained constant.

COMMENT

This man, whilst he received 670 mg. of quinine sulphate daily, was allowed to experience a severe and prolonged attack of primary vivax malaria.

* Q.A.P. quinine sulphate (as a mixture with ac. sulph. dil.) 670 mg. (10 grains) t.d.s. for 3 days, atabrin dihydrochloride 600 mg., 500 mg., 400 mg., 300 mg., 200 mg. on 5 consecutive days, quinine sulphate (as a mixture with ac. sulph. dil.) 330 mg. with plasmoquine naphthoate 20 mg. t.d.s. for 5 days.

before he received treatment. Subsequently a secondary attack developed and treatment was delayed for some 5 weeks.

In spite of the fact that demonstrable trophozoites were present for some 52 days (day +13 to day +65), this man developed slight tolerance to his infection—there was no significant change in his temperature response though his general reaction diminished and became stable after the first 5 to 6 weeks. There was no evidence that he had increasing ability to deal with his trophozoites though the densities varied from day to day.

The clinical features of his secondary attack, however, showed that he had acquired considerable tolerance since his primary attack. His general reaction was very mild and much higher parasite densities were required to provoke a significant increase in his oral temperature. There was a striking difference between the incidence of major symptoms, the degree of splenomegaly and the weight loss in the two attacks. This difference was still more significant when the possible effect of the daily dose of quinine, administered during the primary attack, was taken into account. It should have assisted him in some manner in the primary attack, either by diminishing his reaction or by helping him to deal with his trophozoites.

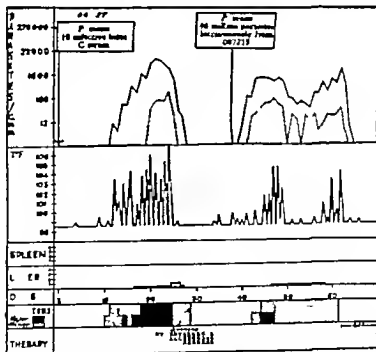


CHART 2.—The development of tolerance in volunteer who received an intravenous inoculum of trophozoites after completing therapy for primary parasite induced malaria (Volunteer 00027 Experiment 7).

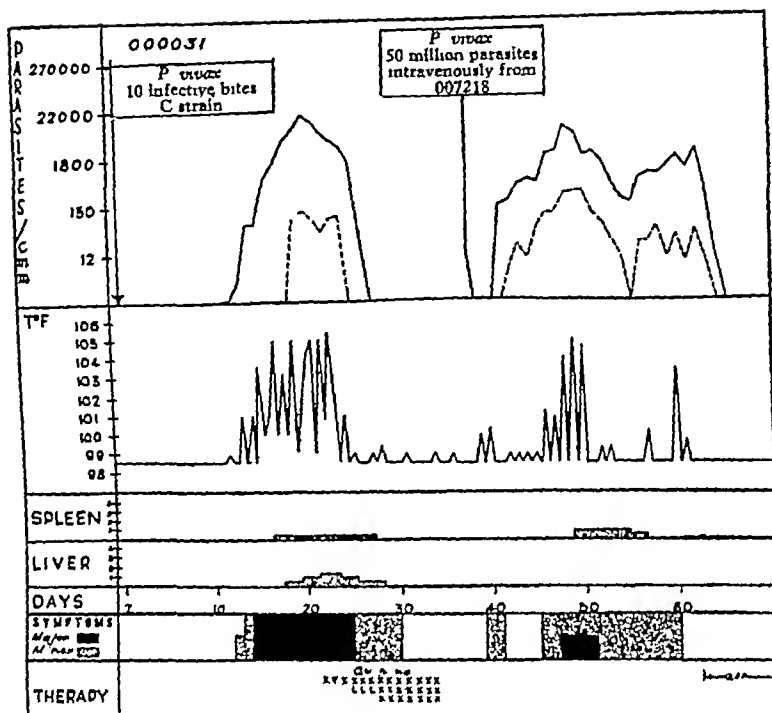


CHART 3—The development of tolerance in a volunteer who received an intravenous inoculum of trophozoites after completing therapy for primary sporozoite induced malaria (Volunteer 000031, Experiment 2)

There was little evidence that he was better able to deal with his trophozoites in the secondary attack, in which the maximum density was 21,100 per c.mm.

It was of interest that the clinical features of the secondary attack were tertian throughout, but in the primary they were essentially quotidian.

Experiment 2 The development of tolerance in two men who received intravenous inocula of trophozoites after completing therapy for primary sporozoite induced malaria (Volunteers 000027 and 000031) (Charts 2 and 3)

Two men, aged 25 and 22 respectively, who had not previously been exposed to malaria, were each bitten by ten *A. punctulatus punctulatus* infected with viable *P. vivax* sporozoites "C" strain (Chart 12), on day 0. Neither man received any drug therapy until he was treated for overt malaria.

The batches of mosquitoes, which fed on both men, were 94 per cent and 95 per cent infected, the salivary gland infections were heavy, and sporozoites had been in the salivary glands for 5 and 7 days respectively.

Trophozoites were first demonstrated in thick blood films collected from both men on day + 12 and the densities increased to reach maxima of 6,900 per c.mm. on day + 21 and 16,800 per c.mm. on day + 20 respectively.

Their oral temperatures exceeded 100° F. for the first time on days + 11 and + 13 respectively and subsequently reached 101° F. to 107° F. each day till full therapy was commenced on day + 24.

Both men developed palpable livers, but only Volunteer 000031 palpable spleen. Symptoms were first remarked on days + 11 and + 12 respectively and were minor till days + 16 and + 15 respectively when both men were confined to bed. They both had quotidian attacks of malaria with such severe symptoms that small doses of quinine sulphate (0.33 or 0.66 grammes) were administered on days + 1 + 22, and + 23 to control them.

Full treatment was given from day + 24 to + 33 and consisted of quinine sulphate (as mixture with ac. sulph. dil.) 2.7 grammes daily for 3 days, followed by quinine sulphate 2.0 grammes daily for 7 days. Quinine was last detected in the plasma collected from these men on day + 35 when the concentration was 0.1 mg. per litre.

Each man was subinoculated on day + 38 with positive results. The two recipients of 200 ml. of their whole blood developed attacks of vivax malaria which were of normal severity and required therapy. Five minutes after the subinoculations were finished, each donor received an intravenous inoculum of blood containing 50.6 million and 45.6 million *P. vivax* trophozoites respectively (from Volunteer 007218). The strain of *P. vivax* inoculated was the same as that used for the original sporozoite infection. (See Chart 12.)

Two and 3 days later (days + 40 and + 41 respectively) trophozoites were demonstrated in thick blood films collected from these men. The densities increased to reach maxima of 1,140 per c.mm. and 8,400 per c.mm. on days + 44 and + 48 respectively and then decreased to maxima of 40 per c.mm. and 140 per c.mm. respectively on day + 56. Another trophozoite was then developed in both men and densities of 2,780 per c.mm. and 2,200 per c.mm. were reached on day + 62, when full treatment with standard Q.A.P. was commenced.

First oral temperatures of 100° F. or over were recorded on days + 40 and + 48 respectively (Volunteer 000031 had raised temperature on days + 40 and + 41, which was attributed to large alveolar abscess), and quotidian increases in temperature obtained to days + 50 and + 51 respectively. The changes in oral temperature during the last trophozoite wave were minor and largely erratic. Volunteer 000027 had minor symptoms on days + 43 and + 44 major symptoms between + 45 and + 47 but subsequently symptoms were minor (he was never graded below (c) after day + 47). The reaction of Volunteer 000031 was confused by the previously mentioned alveolar abscess, but major symptoms did not occur until day + 49 and were present till day + 51 after which he was not graded below (b).

Splenomegaly recurred in the volunteer previously showing it, and the other volunteer developed palpable liver margins—in both instances during the trophozoite wave immediately following the intravenous inoculation.

The changes in red blood cell counts, haemoglobin concentrations and body weights are set out below:—

Day	R.b.c. in millions per c.mm.		Hb. in grammes per 100 ml.		Weight in lb.	
	000027	000031	000027	000031	000027	000031
0	5.26	5.10	17.0	18.4	113	120
+ 46—+ 29	3.70	3.76	11.0	10.2	1	122
+ 26—+ 4	4.42	4.43	12.4	12.1	22	120
+ 52—+ 57	3.92	3.46	11	10.2	1.3	118
64					16	115

COMMENT

These two men had severe attacks of primary malaria which were adequately treated with quinine and were clinically cured. On the fifth day after ceasing therapy, when their plasma was cleared of quinine, submicroscopic densities of trophozoites were found by subinoculation to be present in their peripheral circulation. A few minutes after the subinoculations had been completed moderate doses of the same strain of trophozoites (50 million) were injected intravenously into each man. These parasites multiplied rapidly and elicited attacks of malaria caused which were however, milder than the previous primary attacks. The trophozoite wave subsided but were followed by further waves which produced very mild attacks of malaria in both men.

The last trophozoite waves were regarded as the first secondary waves of trophozoites from the original sporozoite induced infections. This interpretation was supported by observation made on two other men included in the experiment. One of these (Volunteer 000130) was subjected to precisely the same experimental procedures and showed the same response (a fever a day 62) as the two men already described. The other (Volunteer 000121) was subjected to the same experimental procedures but received no intravenous inoculum after subinoculation. He developed a secondary malarial attack with first demonstrable trophozoites in thick blood films on day 48 and first major symptoms on day 55, when treatment commenced.

In spite of previous severe and prolonged attacks of primary malaria, and in spite of the presence of proven parasitaemia neither man was able to prevent the development of high trophozoite densities when 50 million trophozoites of the same strain were injected intravenously. However both men had developed some tolerance as they showed considerably less reaction to their intravenous infections than they did to their original primary attack. Their reaction to their third trophozoite waves was still less marked but therapy was commenced too early to determine the full extent of their tolerance. There was a little evidence that their ability to prevent the development of high trophozoite densities was increased in the second and third attacks.

This experiment also showed that subjects who have recently had primary vivax malaria may develop another attack in response to superinfection by the intravenous injection of trophozoites of the same strain, even though there are trophozoites in the circulation at the time of injection. A test of cure based on such superinfection would be fallacious.

This experiment extends JAMES's (1931) finding that patients could be reinfectd with the homologous strain of *P. vivax*, either sporozoites or trophozoites, during the latent period between primary and first secondary attack (recurrence of JAMES). JAMES's patients had no demonstrable parasites in their peripheral blood and his strain of *P. vivax* was characterized by a latent period of 8 months after the primary attack before the first recurrence.

Experiment 4 The development of tolerance and immunity in a subject whose primary attack of sporozoite induced vivax malaria was aborted by the administration of paludrine. (Volunteer 100008 Chart 5.)

A man aged 19 years who had not previously been exposed to malaria, was bitten on day 0 by twenty-one *A. punctulatus punctulatus* infected with viable sporozoites of *P. vivax*. ("C" strain, Chart 12.) The batches of mosquitoes were 88 per cent. and 90 per cent. infected, the gland infection medium and heavy and the sporozoites had been in the salivary glands for 5 and 7 days respectively.

Paludrine 1.0 gramme daily was first administered on day + 9 and continued up to and including day + 22—a total of 14.0 grammes in 14 days.

Trophozoites were first demonstrated in thick blood films collected on day + 41 and the densities slowly increased to reach maximum of 2,320 per c.mm. on day + 53. Gametocytes were first seen on day + 48 and reached maximum of 500 per c.mm. on day + 56. Treatment commenced on day + 58 when the trophozoite density was 1,320 per c.mm., and consisted of paludrine 0.2 gramme, 0.3 gramme, 0.5 gramme on days + 56 + 57 and + 58 respectively and then 1.0 gramme daily for 13 days.

His oral temperature first exceeded 99° F on day + 42, when it was 99.4° F. remained normal from day + 42 till day + 49 when it reached 102.6° F. and reached 101.8° F. to 105° F. each day subsequently till treatment began. His first symptoms were remarked on day + 49 when he was first down-graded from () to (). On day + 50 he had minor symptoms, but was graded (d) or (e) for at least part of each day subsequently till day + 59 when he was graded (e). He had quotidian attack of average severity losing 8 lb. in weight. His haemoglobin concentration decreased from 14.8 grammes per 100 ml. of blood on day + 47 to 11.3 grammes per 100 ml. on day + 65. Trophozoites were last demonstrated on day + 61.

Blood films collected between day + 62 and day + 102 were negative for malaria parasites, but films collected on day + 103 contained trophozoites in density of less than 1 per c.mm. The trophozoite densities slowly increased to reach maximum of 340 per c.mm. on days + 116 and + 120 subsequent to which they gradually diminished till day + 142, when on trophozoites were seen in thick blood films. During the next 25 days on trophozoites were seen in thick blood films and between day + 168 and day + 201 the densities varied between 0 and 16 per c.mm. He was graded () each day from + 61 to + 201 and he was employed as an orderly in the ward. His oral temperature reached 99° F. on day + 88 and 100.6° F. on day + 104 but otherwise it was normal between days + 89 and + 201. His weight varied between 151 and 133 lb. and his haemoglobin concentration varied between 13.0 and 15.2 grammes per 100 ml. of blood.

He was stable and showed little or no reaction to his continued but minor blood stream infection.

On day + 202 he was given an intravenous inoculum of blood containing 300 million *P. vivax* trophozoites of the same strain (from Volunteer 150002, Chart 12). On this day his blood film contained no demonstrable trophozoites or gametocytes. During the next 10 days trophozoites in densities of 0 to 2 per c.mm. were demonstrated but no gametocytes were seen. No symptoms were recorded. he was graded (a) each day and the only oral temperature above 98.6° F. occurred on day + 210 when 100° F. was recorded. On day + 212 he was used in another experiment (see Part II).

His spleen was palpable between days + 49 and + 61 reaching maximum size of one finger-breadth below the left costal margin. His liver was never palpable.

COMMENT

This man's primary malaria attack was undoubtedly aborted by the administration of paludrine on day + 9 and the subsequent 13 days. Trophozoites were first liberated from the c.e. phase in this strain of *P. vivax* between day + 8 and day + 9 (FAIRLEY *et al.* 1947), but in this man the administration

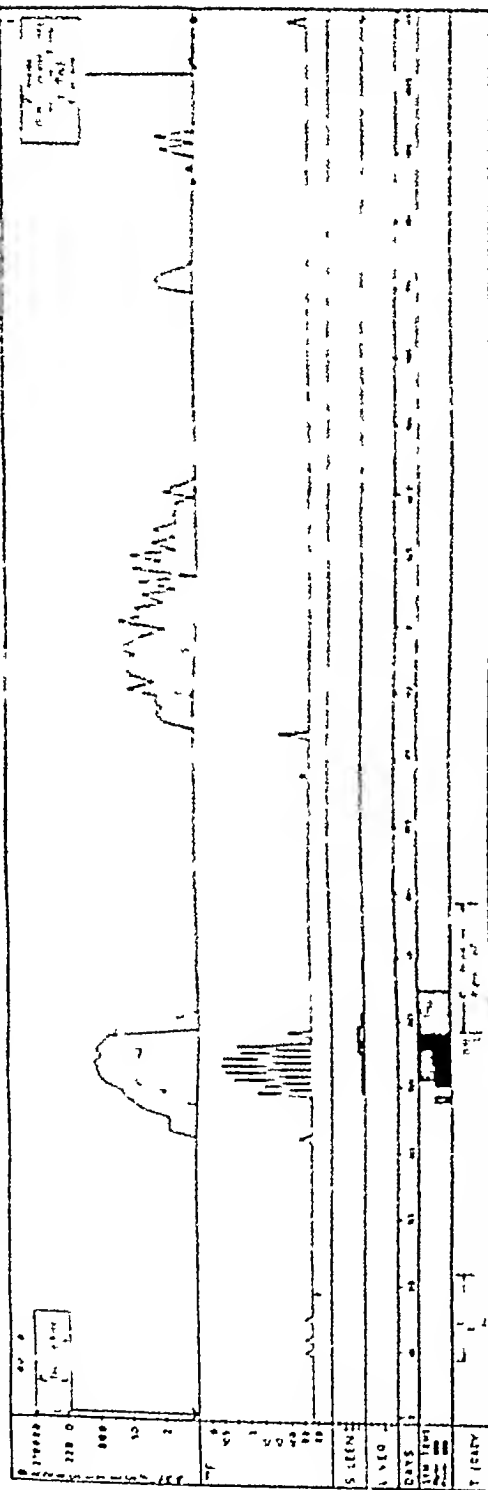


CHART 5—The development of tolerance and immunity in a subject who primary attack of psittacine-induced malaria was aborted by the administration of pyridine (Volunteer James Experiment 1)

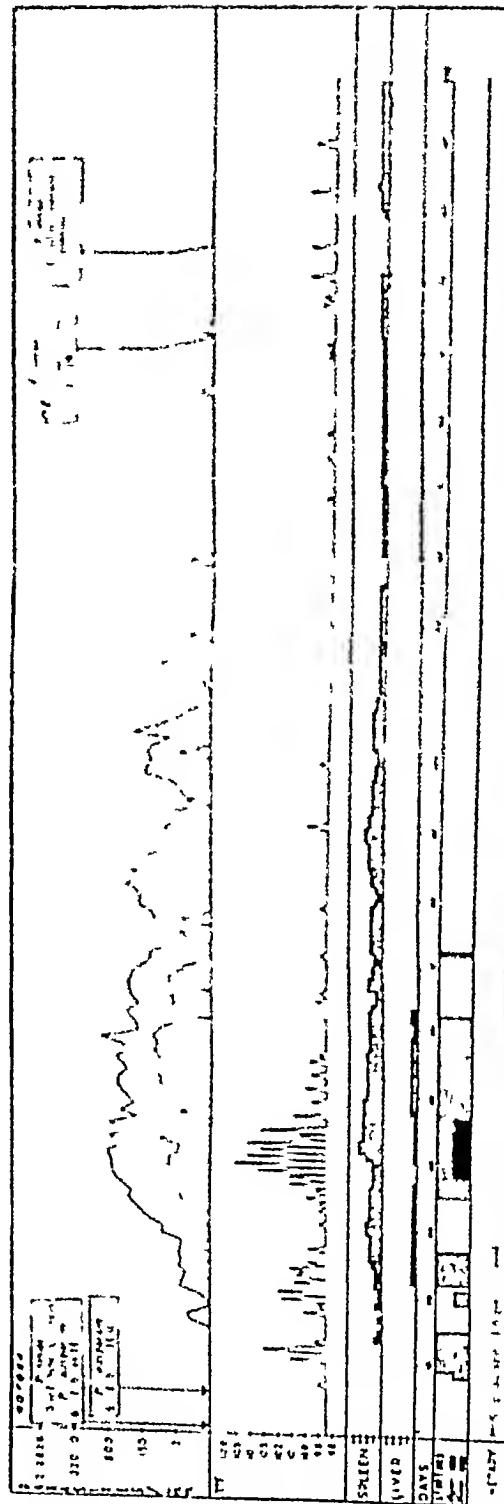


CHART 6—The development of tolerance in a volunteer with psittacine-induced malaria who primary attack was partially suppressed with sulphadiazine, but who never received therapy for overt malaria (Volunteer James Experiment 5)

Experiment 4 The development of tolerance and immunity in a subject whose primary attack of sporozoite induced vivax malaria was aborted by the administration of paludrine. (Volunteer 100009 Chart 5)

A man aged 19 years who had not previously been exposed to malaria was bitten on day 0 by twenty-one *A. punctulatus punctulatus* infected with viable sporozoites of *P. vivax*, (C strain, Chart 12.) The batches of mosquitoes were 80 per cent. and 90 per cent. infected, the gland infections medium and heavy and the sporozoites had been in the salivary glands for 5 and 7 days respectively.

Paludrine 1.0 gramma daily was first administered on day + 9 and continued up to and including day + 22—a total of 14.0 gramma in 14 days.

Trophozoites were first demonstrated in thick blood films collected on day + 41 and the densities slowly increased to reach maximum of 2,320 per c.mm. on day + 53. Gametocytes were first seen on day + 48 and reached maximum of 500 per c.mm. on day + 56. Treatment commenced on day + 56 when the trophozoite density was 1,320 per c.mm., and consisted of paludrine 0.2 gramma, 0.3 gramma, 0.5 gramma on days + 56 + 57 and + 58 respectively and then 1.0 gramma daily for 13 days.

His oral temperature first exceeded 99° F on day + 42, when it was 99.4° F. remained normal from day + 42 till day + 49 when it reached 102.6° F. and reached 101.6° F. to 105° F. each day subsequently till treatment began. His first symptoms were remarked on day + 49 when he was first down-graded from () to (). On day + 50 he had minor symptoms, but was graded (s) or (r) for at least part of each day subsequently till day + 59 when he was graded (). He had quotidian attack of average severity losing 9 lb. in weight. His haemoglobin concentration decreased from 14.6 grammes per 100 ml. of blood on day + 47 to 11.3 grammes per 100 ml. on day + 63. Trophozoites were last demonstrated on day + 61.

Blood films collected between day + 62 and day + 102 were negative for malaria parasites, but films collected on day + 103 contained trophozoites in density of less than 1 per c.mm. The trophozoite densities slowly increased to reach maximum of 340 per c.mm. on days + 116 and + 120 subsequent to which they gradually diminished till day + 14., when on trophozoites were seen in thick blood films. During the next 23 days no trophozoites were seen in thick blood films and between day + 166 and day + 201 the densities varied between 0 and 16 per c.mm. He was graded (s) each day from + 66 to + 201 and he was employed as an orderly in the ward. His oral temperature reached 99° F on day + 98 and 100.6° F on day + 104 but otherwise it was normal between days + 59 and + 201. His weight varied between 131 and 133 lb. and his haemoglobin concentration varied between 13.0 and 16.2 grammes per 100 ml. of blood.

He was stable and showed little or no reaction to his continued but minor blood stream infection.

On day + 202 he was given an intravenous inoculum of blood containing 200 million *P. vivax* trophozoites of the same strain (from Volunteer 150002, Chart 12). On this day his blood film contained no demonstrable trophozoites or gametocytes. During the next 10 days trophozoites in densities of 0 to 2 per c.mm. were demonstrated but no gametocytes were seen. No symptoms were recorded; he was graded () each day and the only oral temperature above 98.6° F occurred on day + 210 when 100° F was recorded. On day + 212 he was used in another experiment (see Part II).

His spleen was palpable between days + 49 and + 61 reaching maximum size of one finger-breadth below the left costal margin. His liver was never palpable.

COMMENT

This man's primary malaria attack was undoubtedly aborted by the administration of paludrine on day + 9 and the subsequent 13 days. Trophozoites were first liberated from the e.e. phase in this strain of *P. vivax* between day + 8 and day + 9 (FAIRLEY *et al.*, 1947), but in this man the administration

of paludrine prevented their multiplication to a density that could be demonstrated in thick blood films.

His first secondary attack began on day + 41 and was marked by considerable constitutional disturbance, but his trophozoite densities did not reach as high levels as was usual in other volunteers used in similar experiments. There was little evidence of tolerance and the low parasite densities could not necessarily be taken to indicate a degree of "natural" immunity.

His second secondary attack was characterized by a small trophozoite wave without any constitutional disturbance. Trophozoite densities never reached the levels observed in his first attack when symptoms first occurred. He became stabilized to minimal parasite densities during the next 99 days, and was then heavily reinfected (200 million trophozoites intravenously) with the same strain. There was no clinical or parasitological reaction to this inoculum—he appeared to be immune to this strain of *P. vivax*.

It is noticeable that during the phase of balance between his trophozoite densities and his resistance his haemoglobin concentration was normal, his spleen and liver were impalpable, he appeared to be perfectly normal on physical examination, and gametocytes were not seen in thick blood films. He was used as an orderly during this period and on more than one occasion over-indulged in alcohol without reaction beyond that expected in a normal person.

Experiment 5. The development of tolerance in a volunteer with sporozoite induced vivax malaria, whose primary attack was partially suppressed with sulphadiazine but who never received therapy for overt malaria. (Volunteer 007084 Chart 6.)

A 24-year-old man, who had not previously been exposed to malaria, received an intravenous inoculation of 200 ml. of whole blood from test volunteer 63 days before being used in the present experiment. This test volunteer had been exposed to *P. falciparum* sporozoites transmitted malaria 44 days previously, but his infection had been suppressed and cured by the administration of antochin (SN 6011) 100 mg. daily from 2 days before infection to 23 days after infection. Neither volunteer developed any evidence of infection with malarial parasites.

On day 0 of the present experiment he was exposed to ten *A. punctulatus punctulatus* infected with *P. vivax* sporozoites ("C" strain, Chart 12) and six *A. punctulatus punctulatus* infected with sporozoites of *P. falciparum* and on day + 6 he was again exposed to six infective bites from the *P. falciparum* batch. The batch of mosquitoes infected with *P. vivax* was 100 per cent. infected, the gland infections heavy and the sporozoites had been in the salivary glands for 3 days. The *P. falciparum* infected mosquitoes were 100 per cent. infected, the gland infections heavy and the sporozoites had been in the salivary glands for 11 days at the first exposure and 17 days at the second exposure.

Sulphadiazine, 1.0 grammes daily was administered from day — to day + 23 inclusive. (Le from 2 days before the first exposure up to and including 22 days after the last exposure to infection.)

Neither trophozoites nor gametocytes of *P. falciparum* were ever demonstrated in thick blood films collected from this volunteer neither during the period of sulphadiazine suppression nor after its cessation.

The first *P. vivax* trophozoite was demonstrated in thick blood films collected on day + 10, when only one was seen but more were seen during the next 5 days. Between days + 15 and + 28 when the administration of sulphadiazine ceased, trophozoite densities varied from 0 or less than 1 per c.mm. to 10 per c.mm.

During this period he had some headache and malaise but was never graded below (b) or (c), which were his usual gradings. His spleen was first palpable below the costal margin on day + 14 and reached two finger-breadths on day + 26. His oral temperature reached 101°F on day + 10, 103°F on day + 11, became normal for 3 days, and then ranged between 99°F and 102°F till day + 26, after which it was normal for 3 days. His haemoglobin concentration fell from 17.2 grammes per 100 ml on day - 2 to 13.4 grammes per 100 ml on day + 32.

Subsequent to ceasing suppression, trophozoite densities in his peripheral blood increased to reach a maximum of 1,740 per c mm. on day + 44. After this they gradually diminished in an irregular fashion to reach 0 on day + 108, and negative blood films were consistently obtained between days + 122 and + 128. After days + 128 and + 129, when the densities were 1 and 6 per c mm. respectively, only an occasional trophozoite was seen in thick blood films till day + 161, when further experiments were performed.

Coincident with his increasing trophozoite densities, he developed major symptoms and had several shivers and sweats, though he was symptom free between days + 28 and + 35, when he was graded (a). After day + 35 he was graded (d) or (e) for a part of each day till day + 47. His oral temperature reached 100°F on day + 35 and readings of 100.4°F to 104.6°F were obtained each day till day + 48, subsequent to which he had tertian rises of 100°F to 100.4°F for 10 days. During this tertian period his symptoms were minor but showed little tertian change. His haemoglobin concentration decreased to a minimum figure of 11.2 grammes per 100 ml on day + 46, his spleen reached a maximum size of 3.5 finger-breadths below the left costal margin on day + 42 but gradually diminished in size after this till, by day + 161, it was a little less than one finger-breadth below the left costal margin.

Between day + 58 and + 161 there were no symptoms and he was graded (a), but occasional temperatures of 99°F were recorded. His haemoglobin concentration increased to reach 18.7 grammes per 100 ml on day + 160, when his red blood cell count was 6.24 million per c mm.

On day + 144, when only one trophozoite could be found in an examination of 3 c mm of blood in thick films, 1 ml of blood was taken from his left cubital vein and immediately transferred intravenously into Volunteer 051004. This recipient developed a typical attack of vivax malaria—trophozoites were first demonstrated in thick blood films collected 6 days after the inoculation and the densities increased to reach 7,000 per c mm by the 13th day, he developed minor and then major symptoms, being graded (a-d) on the 11th day though he had an oral temperature of 103.4°F on the ninth day.

Seventy-two ml of blood, containing 475 million trophozoites, were taken from the above-mentioned recipient (Volunteer 051004) on day + 161 and given immediately to Volunteer 007084, the subject of this experiment. During the next 13 days there was no significant reaction to the inoculum. The parasite densities were as follows: on day + 161 (immediately after the injection), 74 per c mm; day + 162, 7 per c mm; day + 163, less than 1 per c mm; day + 164, less than 1 per c mm; and only one trophozoite was seen in the next 10 days. The only oral temperature of over 99°F was one of 100°F on day + 171. He was graded (a) during this period, but there appeared to be a slight increase in the size of his spleen.

On day + 174 he received an intravenous injection of blood containing 400 million trophozoites from Volunteer 100017, these trophozoites were of the same strain (see Chart 12). During the next 25 days parasites were found on three occasions only—on day + 174 (30 minutes after receiving the inoculum) 15 per c mm., and less than 1 per c mm on days + 175 and + 194. There was no significant rise in oral temperature, no symptoms were recorded, and he was graded (a) each day. His spleen remained palpable.

On day - 194 an attempt was made to induce a clinical attack of malaria by exposing him to cold. He was placed in a refrigerated chamber for 1 hour at a temperature of 12°F (-11°C) wearing trousers and boots but no shirt or other clothing and his movements were strictly limited. There was no clinical or parasitological evidence of active malaria during the following 3 weeks.

On day + 188 his haemoglobin concentration was 19.0 grammes per 100 ml., his red blood cell count 6.40 million per c.mm., his total leucocytes 8,300 per c.mm. with 67 per cent. neutrophil granulocytes, 30 per cent. lymphocytes 5 per cent. monocytes, 1 per cent. eosinophil granulocytes and 2 per cent. basophil granulocytes. His weight was 140 lb. prior to beginning the experiment and 143 lb. on day + 194.

N gametocytes were seen in thick blood films collected between days + 107 and + 200.

COMMENT

Although this volunteer was not a "virgin," the blood inoculation he received before being used in this experiment clearly contained no malarial parasites as neither he nor his donor developed any evidence of *P. falciparum* malaria.

The sporozoite induced *P. falciparum* infection was a part of another experiment, and it was completely suppressed and cured by the daily dose of sulphadiazine (1.0 gramme). This result was constantly obtained in similar experiments reported elsewhere. He had a small falciparum trophozoite wave that never reached proportions sufficient to demonstrate in thick blood films.

His primary vivax infection was partly controlled by sulphadiazine but subsequently he developed a typical attack of vivax malaria though treatment was not given. He had developed some tolerance to his infection while receiving sulphadiazine and the rather low maximum trophozoite density (1740 per c.mm.) may have been due to some antiparasitic immunity.

All his symptoms disappeared by day + 58 though trophozoites were constantly present till day + 122. He developed tolerance much earlier than immunity.

The subinoculation of 1 ml. of blood (containing approximately 300 trophozoites) into a recipient showed that the parasites had not been altered—the recipient developed a typical attack of malaria. Massive reinfection with 475 million parasites from this recipient failed to produce any disturbance of his balance. He was immune to his own strain.

Massive reinfection was repeated with the same strain of *P. vivax* obtained from another volunteer again there was no clinical or parasitological reaction.

Finally an attempt was made to induce an attack by chilling but this was unsuccessful.

This volunteer had developed a solid immunity to this strain of *P. vivax*. Like the previous volunteer he showed no clinical evidence of malaria when immunity had developed—his spleen became impalpable, his haemoglobin concentration increased to 19.0 grammes per 100 ml., and he was perfectly fit though *P. vivax* trophozoites could be demonstrated in his blood whenever a daily search was made. Gametocytes could not, however be demonstrated.

Experiment 6. Three volunteers, infected at the same time, who showed the effect of trophozoite experience in the development of tolerance and immunity. One volunteer's infection was suppressed with atebirin (100 mg. daily) for 147 days, one volunteer was given quinine therapy for each clinical

attack, but received no other drug treatment, and the third volunteer received no therapy at any time (Volunteers 014013, 000039 and 000016)

Volunteer 014013 (Chart 7), a 22-year-old man, who had an old arthrodesis of his right knee joint following a septic arthritis at the age of 15 years, had not previously been exposed to malaria. He was given atebirin dihydrochloride 400 mg daily for 4 days and then 100 mg daily from day 0 to day + 147. His mean plasma atebirin level was 25.5 micrograms per litre (36 observations), the highest reading was 38 micrograms per litre, the lowest 19 micrograms per litre, and the standard deviation was ± 1.03 . On day 0 he was bitten by 50 *A. punctulatus punctulatus* infected with sporozoites of *P. vivax* ("C" strain, Chart 12), the batches used were 68 per cent, 74 per cent and 87 per cent infected respectively, the gland infections light, medium and heavy, and the sporozoites had been in the salivary glands 6, 7 and 7 days respectively.

During the next 21 weeks trophozoites and gametocytes were never demonstrated in thick blood films which were collected daily from day + 9 to + 12, and at least each fourth day thereafter (1 c mm. of blood examined on each occasion). He had no symptoms referable to malaria infection, though he had mild upper respiratory tract infections and a head injury during this 21-week period. He was only graded less than (a) with the previously mentioned incidental occurrences. His haemoglobin concentration varied between 15.9 and 18.5 grammes per 100 ml but showed no tendency to fall—it was 18.0 grammes per 100 ml on day — 5 and 18.5 grammes per 100 ml on day + 138. His oral temperature rose to 99° F on many occasions but there was nothing to suggest malaria activity at these times—similar rises in temperature had been observed in this volunteer prior to exposure to infection.

Subinoculation of 20 ml of blood were made on days + 10, + 13, + 18, and then each fourth day after this till day + 142, when the last subinoculation was made. Positive subinoculations were obtained on days + 10, + 13 and + 42, but all the others were negative as the recipients showed no evidence of malaria infection. The three recipients who developed malaria had typical attacks with increasing parasite densities and major symptoms. They all required treatment.

On day + 178, 31 days after ceasing atebirin suppression, trophozoites were first demonstrated in thick blood films. During the first 6 days the densities reached a maximum of 9 per c mm, but by day + 188 had reached 10,640 per c mm. Gametocytes were first seen on day + 185 and reached a maximum of 560 per c mm on day + 189. Treatment commenced on day + 185 and reached a maximum of 10,000 per c mm. His plasma atebirin concentration had constantly been 0 microgram per litre after day + 169.

First symptoms were recorded on day + 179 and became major by day + 181, when he was graded (a-d). He had an attack of average severity with a major paroxysm on day + 189. The attack was essentially tertian and he was graded (d) or (e) for at least a part of days + 181, + 183, + 185, + 187 and + 189. He lost 9 lb in weight during the attack (158 lb to 149 lb) and his haemoglobin concentration fell from 18.5 grammes per 100 ml on day + 168 to 16.1 grammes per 100 ml on day + 189, and 13.7 grammes per 100 ml on day + 196. His spleen was just palpable on day + 185 and remained so for 6 days. His oral temperature first exceeded 100° F on day + 180 and reached 101 to 104.4° F on days + 181, + 183, + 185, + 187 and + 189. Treatment consisted of quinine sulphate (as a mixture), 2.0 gramme daily for 3 days, atebirin dihydrochloride, 600 mg daily for 2 days, atebirin dihydrochloride, 300 mg daily for 3 days, and then atebirin dihydrochloride, 200 mg, with plasmoquin naphthoate, 20 mg daily, for 3 days. This intensive course was followed by atebirin dihydrochloride, 100 mg daily for 42 days as a maintenance course. The entire course of therapy ended on day + 241.

He was transferred to another hospital (in a non-malarial area) on day + 218, as he developed evidence of reactivation of his old infection in his right knee—a result of an injury sustained whilst swimming in a creek. He developed an attack of clinical malaria,

with demonstrable *P. vivax* trophozoites in thick blood films on day + 274 and received treatment.

Volunteer 000039 (Chart 5) 22 year-old man, who had not previously been exposed to malaria, was bitten by fifty *A. punctulatus punctulatus* on day 0. The batches of mosquitoes were 74 per cent. and 87 per cent. infected respectively, the gland infections medium and heavy and the sporozoites had been in the salivary glands for 7 days. He received no drug therapy till he was treated for overt malaria.

Trophozoites were first demonstrated in thick blood films collected on the evening of day + 12 and the densities increased to reach maximum of 8,120 per c.mm. on the evening of day + 18. Gametocytes were first seen on day + 16 and reached maximum density of 100 per c.mm. on day + 20. Treatment began on day + 19.

His first complaint of symptoms on day + 11. In the evening he had minor symptoms, but from day + 14 to day + 15 his symptoms increased in severity till he was graded (d) or (e) for the whole of each day between + 15 and + 21. He had many shivering and sweating attacks, he vomited considerably and had severe malaria attack. His spleen was first palpable below the costal margin on day + 16 but was palpated two finger-breadths below the left costal margin on day + 19 and + 22. His haemoglobin concentration fell from 15.7 grammes per 100 ml. on day - 8 to 11.5 grammes per 100 ml. on day + 21.

During this attack his oral temperature showed quotidian rises—the first rise to over 100° F was on day + 13, when his temperature reached 104° F. Between day + 13 and + 21, when treatment began, the maximum daily temperatures were between 102.6° F and 105° F.

Treatment of this attack, and subsequent attacks, consisted of quinine sulphate (as mixture with ac. sulph. dd.) 2.0 grammes daily for 10 consecutive days. The mixture was given in three equally divided doses and its quinine content was constantly checked chemically. H. responded well to treatment and was graded (a) on day + 23 had practically normal temperature after day + 23, and parasites were last demonstrated in thick blood films collected on day + 23. The last dose of quinine was given on day + 28.

H. remained well for the next 16 days but had some minor symptoms on days + 43, + 46 and + 47. Major symptoms were first remarked on day + 48 and he was graded (d) or (e) for at least part of days + 48 to + 53. His oral temperature frequently reached 99° F between day + 28 and + 43, but on day + 44 temperature of 100.4° F was recorded. Subsequently he had daily rises of temperature reaching 105.6° F on day + 51. Though his spleen diminished greatly in size following treatment of his primary malaria attack, it again enlarged to reach maximum size of two finger-breadths below the costal margin on day + 50.

Blood films were negative each day from + 24 to + 41 but on day + 42 trophozoites were again demonstrated and the densities increased to reach maximum of 8,200 per c.mm. on day + 51. Gametocytes were first demonstrated in thick blood films collected on day + 46 and reached a maximum density of 440 per c.mm. on day + 49. This attack was as severe as the primary attack and treatment with quinine sulphate (in precisely the same dosage) was given from day + 51 to day + 60 inclusive.

His response to treatment was good, he was graded (a) on day + 57 the last parasites were demonstrated in thick blood films collected on day + 54 and his oral temperatures did not reach or exceed 100° F after day + 52.

In this attack there was fall in haemoglobin concentration—on day + 42 it was 14.3 grammes per 100 ml., but it was 10.6 grammes per 100 ml. on day + 56.

Blood films were negative till day + 69 when trophozoites were again found. The densities increased to reach maximum of 7,800 per c.mm. on day + 81 gametocytes were first demonstrated on day + 73 and the maximum density was 220 per c.mm. on day + 81.

H. was graded (a) from day + 57 to day + 77 but minor symptoms were present from + 78 to + 79 and he was graded (d) or (e) for part of days + 80 and + 82. His clinical attack was not so severe as the previous two attacks. His oral temperature occasionally reached 99° F between days + 83 and + 77 but rose to 101.4° F on day + 78 following which there were irregular tertian rises till day + 83 when treatment began.

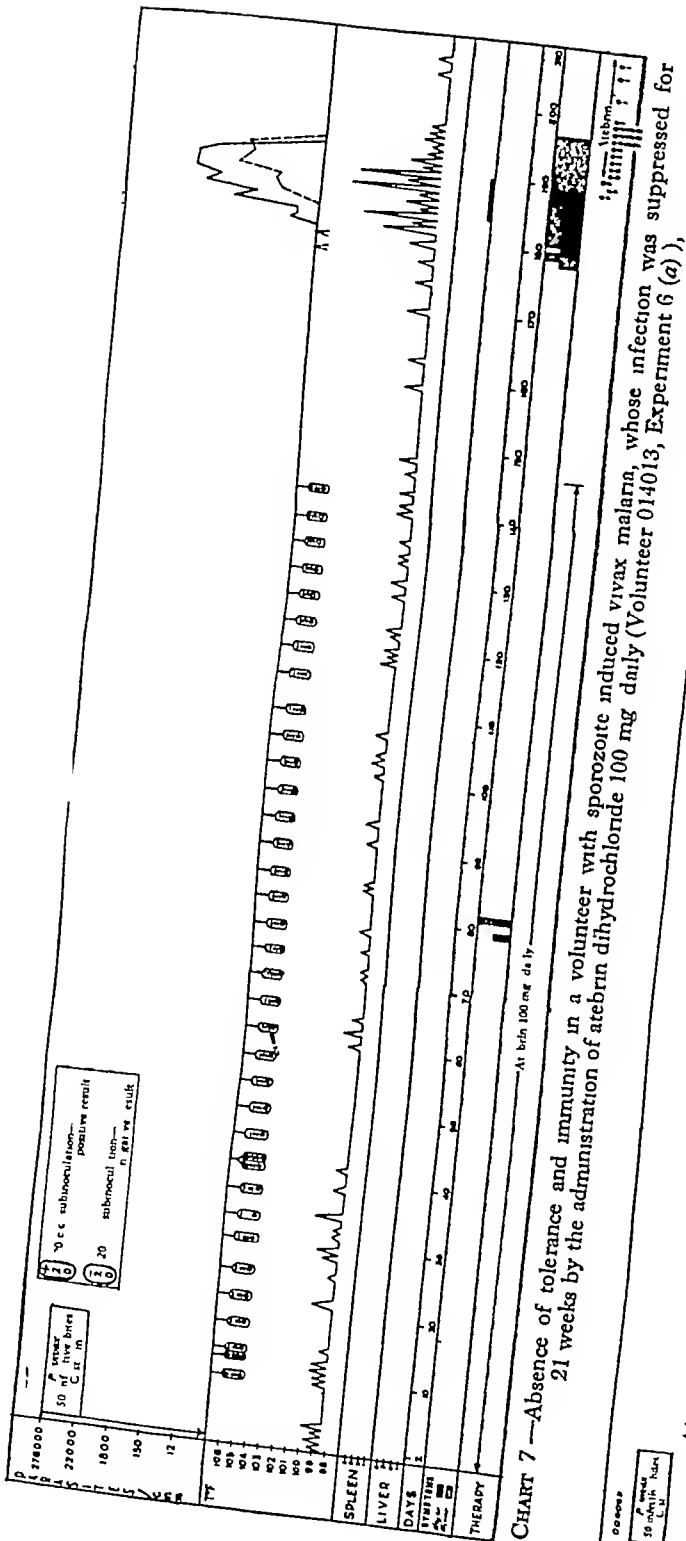


CHART 7—Absence of tolerance and immunity in a volunteer with sporozoite induced vivax malaria, whose infection was suppressed for 21 weeks by the administration of atebn dihydrochloride 100 mg daily (Volunteer 014013, Experiment 6 (a)),

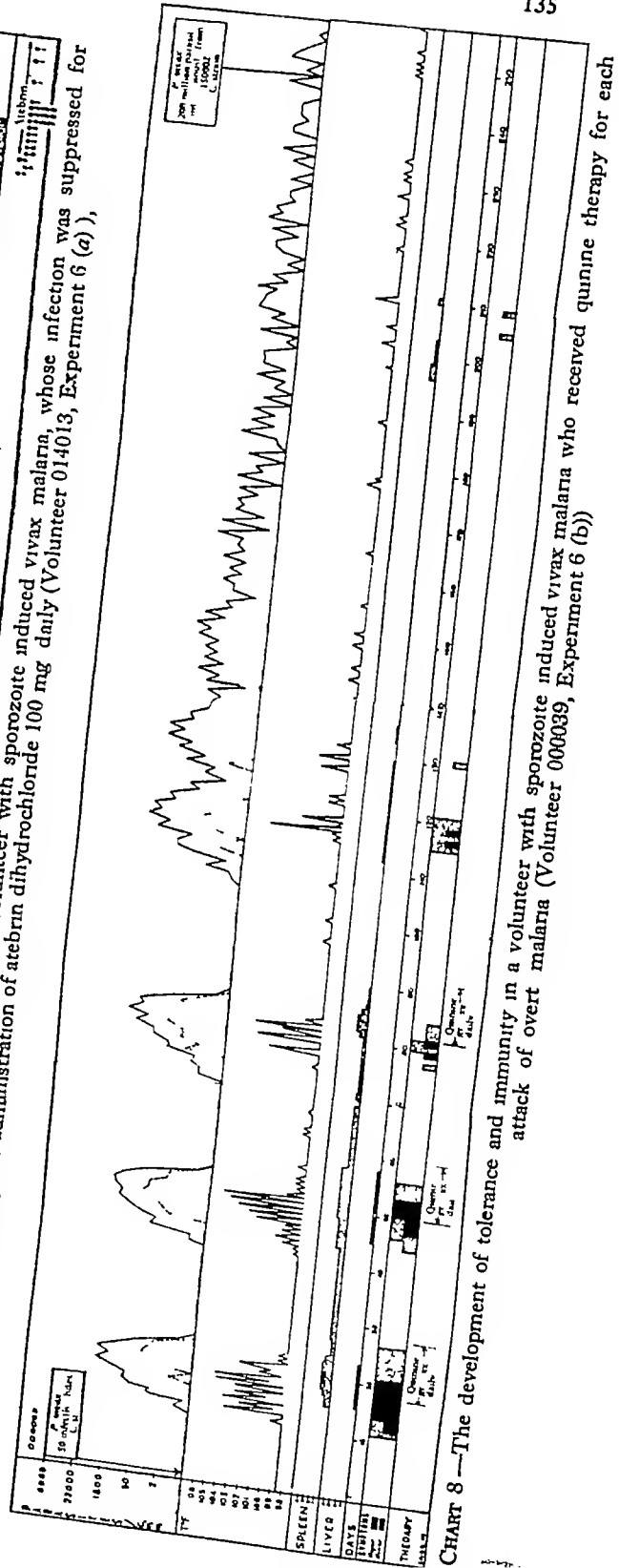


CHART 8—The development of tolerance and immunity in a volunteer with sporozoite induced vivax malaria who received quinine therapy for each attack of overt malaria (Volunteer 000039, Experiment 6 (b))

His spleen, which had diminished in size after his second attack, now enlarged again and reached to two finger-breadths below the left costal margin between days + 83 and + 85. His haemoglobin concentration fell from 13.5 grammes per 100 ml. on day + 70 to 8.9 grammes per 100 ml. on day + 84.

Quinine sulphate, in the same dosage, was given again for 10 days from day + 83 to day + 92. His response to treatment was rapid, parasites were last demonstrated in blood films collected on day + 85, he was graded (a) on day + 86 and his oral temperatures did not reach or exceed 100° F. after day + 83. He remained well and had no oral temperature of 100° F. or more till day + 117. Blood films collected each day between day + 84 and + 105 were negative but trophozoites were demonstrated on day + 106. The densities increased to reach maximum of 6,500 per c.mm. on day + 116. He was graded (a) from day + 86 to + 107, (b) from day + 108 to + 118 (a-e) on days + 117 and + 119 and (b) on + 118 + 120, and + 121. In this mild attack there was little if any change in spleen size symptoms were never severe, and treatment was not given.

Trophozoite densities tended to diminish between days + 116 and + 123, but then another small wave occurred with maximum of 2,500 per c.mm. on day + 130. This small trophozoite wave was associated with minor clinical reaction.

His trophozoite densities then settled down slowly to reach densities between 4 per c.mm. and 50 per c.mm., with occasional negative blood films, between days + 185 and + 247 when other experimental procedures were instituted. He was quite fit in this period and was graded () from day + 132 to day + 247 with the exception of parts of days + 206 and + 210 when he was graded (b). Only once during this period did his oral temperature reach or exceed 100° F. and that was on day + 210. This slight rise in temperature and minor symptoms of malaise and headaches were not associated with an increase in the trophozoite density in the peripheral blood.

His haemoglobin concentration, which was 8.9 grammes at the height of his third attack on day + 84 had decreased to 14.2 grammes per 100 ml. by day + 115 diminished to reach minimum of 11.1 grammes per 100 ml. on day + 124 but subsequently increased to vary between 15.4 and 16.1 grammes per 100 ml. between days + 218 and + 245.

His spleen was occasionally just palpable below the left costal margin between days + 81 and + 185 was definitely palpable between days + 196 and + 202, but was subsequently impalpable.

On day + 247 he was given an intravenous inoculum of 10 c.c. whole blood from Volunteer 160002 (Chart 12) which contained 200 million trophozoites of the same strain as had been used for his initial infection. No symptoms occurred and he was graded () each day subsequently till other experimental procedures were used 10 days later. His oral temperatures showed no change, and no increase in trophozoite densities was observed in his peripheral blood. His spleen did not increase in size. No reaction to this inoculum could be detected, either clinically or parasitologically. Gametocytes were not seen in any thick blood films collected after day + 128 that is over period of some 130 days.

Volunteer 000018 (Chart 9) 22 year-old man who had not previously been exposed to malaria, was bitten by fifty *A. punctulatus punctulatus* infected with *P. vivax* sporozoites (Strain C, Chart 12) on day 0. The batches of mosquitoes were 87 per cent. and 68 per cent. infected, the gland infections heavy and light respectively and the sporozoites had been in the salivary glands for 7 and 8 days respectively.

This man received no anti-malarial drugs at any time either prior to or during the course of this experiment.

Trophozoites were first demonstrated in thick blood films collected on the morning of day + 11 and the densities increased to reach maximum of 10,300 per c.mm. on the afternoon of day + 18. Gametocytes were first seen on day + 18 and reached a maximum of 1,300 per c.mm. on day + 27. From day + 18 to day + 33 the trophozoite densities decreased to reach minimum of 610 per c.mm. on day + 33.

Symptoms were first remarked on day + 12, though an oral temperature of 99° F. was first recorded on day - 11 and he rapidly developed severe attack of primary malaria, being graded (a) for at least part of each day from + 13 to + 19 and on day

+ 22 but (e) for all of days + 21, and + 23 to + 27. He had severe shivering and sweating attacks between the 19th and 24th days inclusive.

His oral temperature first exceeded 100°F on day + 12, when it was 101.6°F , between days + 13 and + 26 his oral temperature reached 103.2°F to 106°F each day, then showed minor tertian tendencies till day + 33.

His spleen was first palpable below the left costal margin on day + 17 and was two finger-breadths below it on day + 25. His liver was tender but not palpable.

Five days before infection his haemoglobin was 17.0 grammes per 100 ml, on day + 12 it was 17.6 grammes per 100 ml, on day + 20 14.5 grammes per 100 ml, and by day + 32 it had fallen to 10.2 grammes per 100 ml—a fall of some 7 grammes per 100 ml. During this period he lost a little over 9 lb in weight.

From day + 33 to day + 36 (inclusive) trophozoite densities remained between 610 and 720 per c mm there were only slight rises in oral temperature, and he was classified (b) or (c).

On day + 37 his trophozoite densities increased to 1,200 per c mm, and then ranged between 1,300 and 2,600 per c mm for the next 3 weeks (till day + 58). Following this the densities declined to reach 24 per c mm on day + 103. Gametocytes declined in numbers from day + 27, when the maximum was observed and were not seen after day + 73.

His oral temperatures showed a daily rise to 100.8°F to 104.6°F between days + 37 and + 46, subsequently showing diminishing daily rises till day + 74, after which an oral temperature of 99°F was recorded on four occasions up to day + 103.

Corresponding with the increasing trophozoite densities, his symptoms increased and he was classified (d) or (e) for a part of each day from + 39 to + 50, but after this his symptoms diminished so that he was classified (a) each day after + 77. He had major paroxysms on days + 42 and + 43, with minor rigors on many other days.

His spleen reached its maximum size of a little over two finger-breadths below the left costal margin on day + 41 and remained about this size till day + 77, when it began to diminish in size, but it remained palpable till day + 103. His liver became tender whenever he had severe symptoms and it was palpable one finger-breadth below the costal margin on day + 70.

His haemoglobin concentration reached a minimum of 8.1 grammes per 100 ml on day + 63, but increased thereafter to reach 12.8 grammes per 100 ml on day + 98. He received no iron or other anti-anaemic therapy.

He was absent from Cairns from day + 104 to day + 131 to attend urgent family matters associated with his father's death. During this period he remained well and carried on a normal existence in a country town in winter. He had no anti-malarial therapy nor any therapy directed against his anaemia.

On his return he was found to have improved—his trophozoite densities were now 1 to 5 per c mm, his haemoglobin concentration 15.4 grammes per 100 ml, he had no symptoms and was graded (a). His spleen was unpalpable.

Between days + 132 and + 277 parasites were occasionally demonstrated in thick blood films, but after day + 154 the maximum number was 2 per c mm. He had no symptoms and was graded (a) each day, his haemoglobin concentration ranged between 15.4 and 18.0 grammes per 100 ml (average of fifteen observations = 16.5 grammes per 100 ml). His spleen and liver were unpalpable and he was employed at various tasks in the unit. His oral temperature occasionally reached 99°F , but there were no significant changes. Gametocytes were last seen in thick blood films collected on day + 86, none was seen during the next 171 days.

On day + 247 he was given an intravenous inoculation of blood containing 200 million trophozoites (strain 'C') in 20 ml of blood (from Volunteer 150002, Chart 12). Following this injection two trophozoites were seen in 1 c mm of blood collected on day + 249, but not on any other day. He developed no symptoms was graded (a) each day, and had no increase in oral temperature.

On day + 257 he was used in another experiment.

COMMENT

These three volunteers were given a heavy sporozoite infection on the same evening from the same batches of mosquitoes. The first volunteer received atebirin 100 mg daily for 21 weeks, the second was given quinine treatment for each attack of overt malaria, reaching the usual degree of severity requiring therapy and the third received no treatment at any time. Observations were made over periods of 218 to 256 days.

Though the first volunteer (014013) was proved to have had two minor trophozoite waves (shown by positive subinoculations on days +10 +13 and +42) and though e.e. forms of *P. vivax* had existed in his tissues for some 178 days, he developed a severe attack of malaria after ceasing atebirin suppression. His trophozoites built up to a density of 10,640 per c.mm. before treatment was given. Furthermore, after receiving quinine, atebirin and plasmoquine treatment, which was followed by a 6 weeks course of atebirin 100 mg daily he developed another attack of malaria which required treatment on day +274. He showed little evidence of either tolerance or anti-parasitic immunity in his first attack and in his second attack neither of these could have been well marked as he required treatment. It was apparent that, in this volunteer the e.e. phase did not produce any significant change in his reaction—the small degree of tolerance could equally well be related to the minor trophozoite waves.

The second volunteer (000039) showed increasing tolerance to his infection as his symptoms and signs developed at progressively later stages and at higher trophozoite densities in each succeeding malaria attack. The table below clearly shows these phenomena during the acquisition of tolerance.

Attack number	Trophozoite densities per c.mm. and day of trophozoites was when first classical features were recorded.				Oral temperature 100° F or more
	Minor symptoms		Major symptoms		
1	0	4 (1)	1	25 (7)	2 3 (2)
2	110	478 (4)	~800	(7)	140 (2)
3	620	2,300 (10)	1 100	7 000 (12)	620 2,300 (10)
4	3	30 (3)	1,800	4,000 (17)	1,800 4 000 (17)

Figures within brackets indicate the day of the demonstrable trophozoite as in each the phenomena etc. observed

A study of the trophozoite densities showed that he developed some anti-parasitic immunity during this period, but it was minor compared with his

tolerance to trophozoites The maximum densities of trophozoites reached in these attacks were —

1st attack,	6,120	per c mm	on the	7th	day of demonstrable parasites
2nd	„	6,200	„	10th	„ „ „
3rd	„	7,600	„	13th	„ „ „
4th	„	6,500	„	11th	„ „ „

In regard to these figures, it must be remembered that treatment was given on the 8th, 10th and 15th days of the trophozoite waves in the first three attacks and none for the fourth. If the attacks are reduced to a common basis by comparing the densities up to and including the eighth day the following maxima are obtained. First attack, 6,120 per c mm, second attack, 2,800 per c mm, third attack, 160 per c mm, and fourth attack, 400 per c mm. Thus he showed some increased ability to prevent his parasites from rapidly reaching high densities. As his fourth attack progressed, his symptoms decreased, and although on days +125 and +127 he had parasite densities of 2,500 and 2,400 per c mm, his lowest grading was (c) on day +125, when his parasites increased from 230 per c mm to 2,500 per c mm. His tolerance outstripped his immunity.

With the gradual subsidence of his trophozoite wave he became perfectly fit and, like the previous two volunteers (Experiments 4 and 5), had a normal haemoglobin concentration and an impalpable spleen. Massive reinfection failed to disturb his equilibrium and served to demonstrate his tolerance and immunity to this strain.

The third volunteer had a long and severe attack of malaria which recrudesced in the 5th week, by which time he had acquired some tolerance—in his first attack he was classified (d) on the third day of his trophozoite wave, when the densities increased from 50 to 130 per c mm, but in the recrudescence he was first classified (d), when the densities increased from 1,200 to 2,660 per c mm on the sixth day (as far as could be judged). His last symptoms were recorded on the 77th day (when his trophozoite densities were about 200 per c mm) and his trophozoites reached insignificant densities on day +138. The failure of massive reinfection to disturb his balance demonstrated his tolerance and immunity. Like the other volunteers with marked tolerance and immunity, there were no signs of malaria infection beyond the occasional demonstration of trophozoites, but no gametocytes, in the peripheral blood stream.

These experiments clearly showed that the development of tolerance to the effects of vivax malaria and of an efficient mechanism for the prevention of the development of great numbers of trophozoites and gametocytes depended on the extent of the man's previous experience of trophozoites. Also the development of tolerance to trophozoites preceded the development of efficient anti-parasitic mechanisms.

In the first experiments, in which the volunteers had but little trophozoite experience, there was minimal ability to prevent the trophozoites from reaching high densities in the peripheral blood stream though the reaction of the individual was considerably lessened. The later experiments showed that after some weeks volunteers could develop tolerance to such a degree that massive reinfection failed to produce any clinical effects and an anti-parasitic mechanism so efficient that hundreds of millions of parasites could be removed from the peripheral blood stream in a day or so.

The last experiment, in which three volunteers were exposed to the same infection on the same night, was of great interest. The volunteer who received atobrin suppression for 21 weeks developed a typical severe attack of malaria after ceasing his suppression. After some 180 days, during which time he was proved to have had two minor trophozoite waves, he had little or no tolerance and no apparent immunity. The persistence of the e.e. phase for this period of time seemed to have little effect on the development of immunity. On the other hand, his fellow volunteers, one of whom had four clinical attacks of malaria, while the other had no treatment for a very prolonged attack, were experiencing no clinical phenomena of malaria infection by this time. All three subjects, presumably had similar e.e. experience during these 6 months.

Of considerable clinical importance were the changes in "fitness", spleen size, and haemoglobin values of these volunteers. Once the stage of complete tolerance had been reached, there were no symptoms, the haemoglobin concentration was normal or above normal, and the spleen returned to its former size. The continued presence of trophozoites in the peripheral circulation caused no appreciable reaction in the host.

As all these experiments were made on volunteers using the (c) strain of *P. vivax* that was maintained for some months in the unit it must be pointed out that originally the strain was obtained fresh from New Guinea (see Chart 12). In the later experiments this strain produced attacks of malaria which could not be distinguished, clinically or parasitologically from the attacks it produced months earlier. One soldier who was naturally infected in New Guinea was included in the experiments as he was known to have a considerable degree of tolerance.

Experiment 7 Tolerance and immunity in a naturally infected subject.

P.K. (subject 800000, Chart 10) man aged about 45 years, had an attack of *P. vivax* malaria in November 1942, whilst serving in New Guinea, and was transferred to non-malarial area.

During the next 9½ months he had nine attacks of vivax malaria, each of which was treated. The first two attacks were treated with quinine sulphate 2.0 grammes daily for 4 days, the next two with quinine sulphate 2.0 grammes daily for 5 days, followed by tebrin dihydrochloride 3.0 mg. daily for 5 days, and the remaining five attacks with quinine sulphate 2.0 grammes daily for 3 days, tebrin dihydrochloride 300 mg. daily for 5 days, quinine sulphate 1.0 grammes daily for 2 days, and then quinine sulphate 1.0 grammes and plasmoquine naphthoate 60 mg. daily for 5 days.

On approximately the 225th day after exposure he came under observation by the author when he was admitted to a base hospital with a severe attack of vivax malaria. He was given the standard course of therapy (as already outlined for the five immediately preceding attacks), under strict supervision, and his urine gave a positive Tanret test on the second day of treatment. His symptoms responded normally, but trophozoite densities in his peripheral blood remained unaltered throughout his treatment. At the end of the course of therapy he was given 670 mg of quinine intravenously on each of two succeeding days with but a slight fall in trophozoite densities. On both of the next 2 days he was given neosalvarsan intravenously in dosage of 400 mg and his blood films became negative and remained so for the succeeding 13 days. Trophozoites were again found in his blood films, but he had no appreciable systemic reaction. Following the administration of intravenous quinine, his blood films again became negative and he was given quinine sulphate (as a mixture) 20 grammes daily for the next 6 weeks, during which time he was asymptomatic and his haemoglobin concentration returned to normal. He was transferred to another hospital. During the next 300 days trophozoites were demonstrated in his blood on numerous occasions in spite of the administration of various sulphonamides, atebryn 100 mg or 400 mg daily, and of standard Q A P therapy and two more injections of neosalvarsan. During this period his spleen was usually recorded as palpable below the left costal margin and his haemoglobin concentration varied from 8.6 to 15.7 grammes per 100 ml.

On approximately the 580th day after his first exposure to malaria (some 520 days after he left a malarious area) he was transferred to Cairns, where he remained for the next 560 days.

On arrival at Cairns he had a palpable spleen and demonstrable *P. vivax* trophozoites in thick blood films, but no fever. During his first 42 days his trophozoite densities varied between 4 per c mm. and 780 per c mm. He was graded (b), but only twice had oral temperatures of 100° F or over. His spleen was constantly palpable.

To study the behaviour of his plasma quinine concentrations, he was given quinine sulphate (as a mixture), 5 grain (330 mg), 10 grain (670 mg), 15 grain (100 grammes) and 20 grain (1.67 grammes), each second day respectively, and then, after a gap of 1 day, 30 grain (2.0 grammes) a day for 3 days. No significant abnormalities were found in his plasma quinine concentrations following these doses.

Just prior to giving the first dose of quinine, on the 63rd day, at Cairns, 100 ml of blood were transfused from him into a volunteer (007139), who had not previously been exposed to malaria infection. P K's blood films became negative for malaria parasites on the day after the dose of 670 mg of quinine was given and remained negative till further experiments were carried out 27 days later.

The recipient (Volunteer 007139, Chart 11) of his blood developed a typical attack of malaria which had to be controlled with small doses of quinine so that trophozoites and gametocytes would be available for further experiments. Trophozoites were first demonstrated on the 5th day after inoculation, reached a maximum of 16,800 per c mm on the 16th day, but in the recrudescence, after the effect of a small dose of quinine had disappeared, the densities reached 51,000 per c mm. Gametocytes were first seen on the 12th day and reached a maximum of 740 per c mm on day + 33, when batches of mosquitoes were infected.

Thirty-one days after giving blood to the recipient, P K (subject 500000) received 130 million trophozoites intravenously from Volunteer 007139 (his own recipient). Following this, he had a slight trophozoite wave, reaching a maximum of 320 per c mm 8 days later, but none was present between the 11th and 18th day. Nineteen days after receiving this intravenous inoculum he was bitten by seven *A. punctulatus punctulatus*, heavily infected with 2-day-old sporozoites derived from the gametocytes of Volunteer 007139 (the recipient of P K's blood). He was bitten by mosquitoes infected with his own strain. No clinical signs of infection were observed. A trophozoite wave beginning the day prior to exposure to infective mosquitoes reached a peak of 32 per c mm 5 days later and had terminated by the 12th day. Between the 13th and 17th day after being bitten he had from 12 to 330 trophozoites per c mm in his peripheral blood, but blood films were negative between the 18th and 46th day.

On approximately day + 740 after the original infection (46 days after he was bitten by mosquitoes infected with his own strain), he received an inoculum containing 140

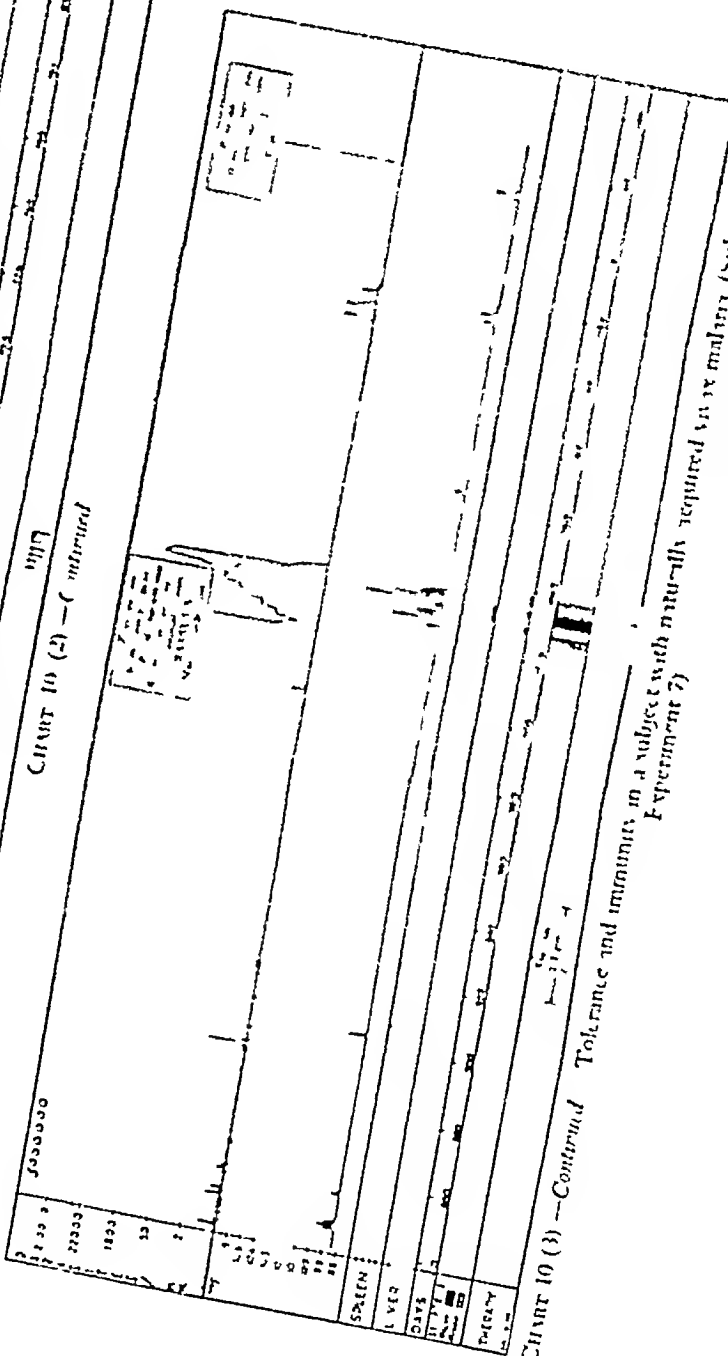


Chart 10 (3) - Continued Tolerance and immunity in a subject with naturally acquired virus infection (continued)

million trophozoites of *P. vivax* ("C" strain, Chart 17) from Volunteer 007018. Following this injection, he developed minor trophozoite waves lasting some 6 weeks, but it reached a maximum density of only 300 per c.mm. on the 14th day.

On about the 780th day he was exposed to 1 *A. punctulatus punctulatus* with medium infection of their salivary glands with 7-day-old sporozoites of "C" strain (from Volunteer 000029 Chart 17). Some 18 days later he had minor trophozoite waves lasting little over 3 weeks in which the maximum density was 140 per c.mm.

Trophozoites were occasionally demonstrated during the next 80 days, but there were no signs of clinical reaction. He was then given course of ateban dihydrochloride, 100 mg. daily for 6 weeks, for the purpose of studying his plasma ateban levels. His blood films became consistently negative and his plasma ateban levels were not abnormal in any way.

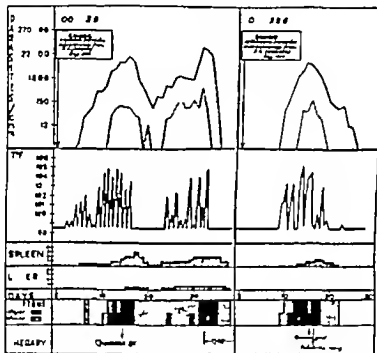


CHART 11—Two recipients of blood containing *P. vivax* trophozoites from Subject 50000 (Volunteer 007139 on day + 635 and Volunteer 007236 on day + 1057)

By approximately the 1000th day after the original infection, he was again showing trophozoites in his peripheral blood stream.

On + 1,020 day 20 ml. of blood were collected from him and injected into the cubital vein of another volunteer who had not previously been exposed to malaria (007226 Chart 11). On this day P.H. had no parasites demonstrable in search of 1 mm. of blood, and it was estimated that the recipient received no more than 20,000 trophozoites in his inoculum. The recipient developed typical attack of vivax malaria with maximum trophozoite density of 11,000 per mm. on the 15th day and maximum gametocyte count of 60 per cm. Therapy was given the next morning. He had considerable pyrexial and symptomatic reaction and his spleen became palpable below the left costal margin. He was used in another experiment on day + 1,030 (see Part II).

COMMENT

This soldier had many attacks of clinical malaria which were associated with demonstrable *P. vivax* in his peripheral blood stream. Ultimately, he became tolerant to his infection, later still he was shown to be immune. Efforts to induce an attack of malaria, either parasitological or clinical, by superinfection with trophozoites or sporozoites of his own strain failed completely. Subsequent attempts to induce an attack using the Cairns "C" strain also failed though trophozoite and sporozoite infections were given. Like the "immune" volunteers previously described, he was fit, had no palpable spleen, and his haemoglobin concentration ranged between 13.0 and 17.2 grammes per 100 ml between days +590 and +1,020.

His *vivax* trophozoites were apparently quite unaffected by long residence in his body—the two subinoculations performed, one on day +635 and the other on day +1,020 were both positive and produced typical severe attacks of malaria in the recipients (Volunteers 007139 and 007226). It was of considerable interest to note that during a period of 430 days at Cairns gametocytes were never seen in thick blood films, but both the recipients of his blood had good gametocyte waves and, where tested, readily infected mosquitoes.

Although the two subinoculations were performed 375 days apart, and although other *vivax* infections had been given to the donor in the meantime, there were no significant differences in the two recipients' attacks. This soldier had developed solid tolerance and immunity to his own strain and to the Cairns "C" strain (they may both have been the same strain).

PART II

AN EXPERIMENT DESIGNED TO SHOW THAT THE DEVELOPMENT OF TOLERANCE PRECEDES THE DEVELOPMENT OF IMMUNITY AND THAT IMMUNITY WAS STRAIN SPECIFIC

In this experiment four men were used, all of whom have already been described in part. They were Volunteer 100009 of Experiment 4, Volunteers 000039 and 000016, of Experiments 6 (b) and 6 (c), and P.K. (500000) of Experiment 7. In Part I the histories of these men ceased at a stage where they were experiencing no reaction to malaria infection, they had normal temperatures, impalpable spleens, normal or above normal haemoglobin concentrations, were graded (a), but all had occasional trophozoites in their peripheral blood streams. The three volunteers (000039, 000016 and 100009) had all been shown to be tolerant and immune to the Cairns "C" strain when given 200 million trophozoites intravenously. The fourth subject was tolerant and immune to both trophozoite and sporozoite transmitted "C" strain *P. vivax*.

Strain "C" was isolated in August, 1944 from a soldier who had served in the south-east areas of New Guinea. During the later stages of the campaign (September 1945), the "A" strain was isolated from a soldier evacuated from the north-east coast (Aitape-Wewak area). The passages of the "C" and

A strain are shown in Chart 12 and the derivation of the trophozoites used in the experiment about to be described can be seen. In volunteers receiving suppressive atebain, and in the recipients of infected blood from the "suppressed" volunteers, this *P. vivax* behaved in a manner indistinguishable from the "C" strain.

Experiment 8.

Ten ml. of blood containing total of 140 million trophozoites of A strain *P. vivax* (from Volunteer 014004) were given to the three volunteers 000009, 000016 and 100009 on the same day (day + 257 for the first two volunteers, and + 228 for the last) [Charts 13 (a) 13 (b) and 13 (c)].

All three volunteers developed overt malaria attacks with demonstrable trophozoites and gametocytes in their peripheral blood stream. Trophozoites were first demonstrated either on the day of the injection or the following day and the densities built up to reach maxima as set out below—

Volunteer 100009: 12,000 per c.mm. on the ninth day (previous maximum, 2,320 per c.mm.).

Volunteer 000009: 27,600 per c.mm. on the eighth day (previous maximum, 7,600 per c.mm.).

Volunteer 000016: 35,300 per c.mm. on the seventh day (previous maximum, 10,900 per c.mm.).

The maximum gametocyte counts were 140 per c.mm., 130 per c.mm. and 320 per c.mm. respectively.

Major symptoms occurred in all three volunteers who were first graded (a) and (a) on days + 7 and + 7 and + 5 at parasite densities of 4,700 per c.mm., 7,000 to 22,000 per c.mm., and 6,880 to 23,600 respectively. Oral temperatures of 100° F. or over were first recorded on the fifth day in all three men at parasite densities of 2,300 per c.mm., 3,300 to 7,050 per c.mm., and 6,800 to 23,600 per c.mm. respectively.

Volunteers 100009 and 000009 were both treated as the severity of their attacks reached the usual therapy level. The former was treated with paludrine and the latter with quinine sulphate 30 grains a day for 10 days (the same therapy used for his previous attacks). Volunteer 000009 had negative blood films from day + 14 to day + 41 when one trophozoite per c.mm. was found. Subsequent films were negative till day + 66 (day + 323 after his original infection), when he was discharged. At the time of discharge he was quite well, graded (a), and his haemoglobin concentration was 14.6 grammes per 100 ml.

Volunteer 000016 received no treatment for his attack and his trophozoite densities decreased in numbers to reach 15 to 30 per c.mm. on the 14th to 16th days after inoculation. Subsequently there were small trophozoite waves reaching maximum of 1,300 per c.mm. on the 23rd day but this had subsided by the 29th day. A still smaller trophozoite wave was then observed, the parasite density reaching maximum of 540 per c.mm. on the 37th day. Subsequently the densities gradually declined to range between 1 to 50 per c.mm. on the 66th day (day + 323) when he was discharged. During this period his haemoglobin concentration fell from an average of 16.5 grammes per 100 ml. to 11.0 grammes per 100 ml. on the 23rd day but was 14.6 grammes per 100 ml. on the day of discharge. Subsequent to the 12th day after inoculation, he was only twice graded less than (a) on the 22nd and 24th days he was graded (b) for part of each day. His spleen was palpable to one finger-breadth below the costal margin on the 11th and 12th days but subsequently was imperceptible.

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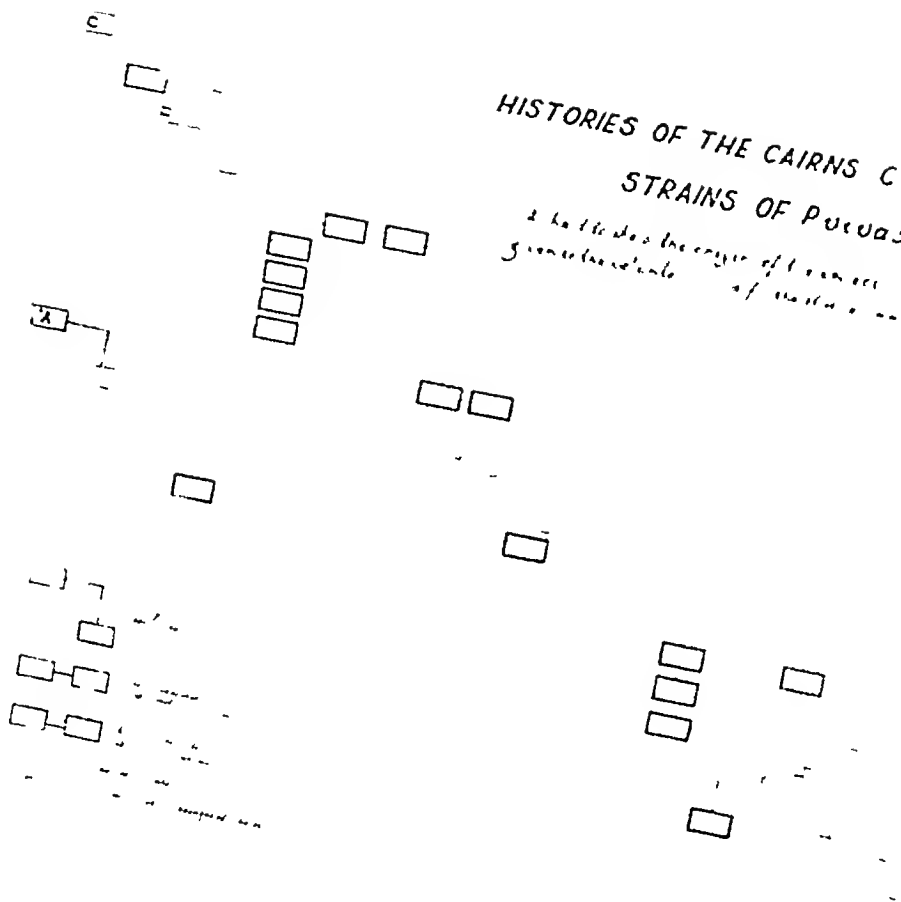


CHART 12



The haemoglobin concentration decreased in all three men, reaching minimal concentrations of 12.8, 10.6 and 11.0 grammes per 100 ml respectively, and all three men lost weight—an average of 6.5 lb in 8 days.

The fourth subject, P K (500000, Chart 10), had a more complicated course. On day + 1,020, immediately after he was last subinoculated (see Experiment 7), he received an intravenous injection of 20 ml of blood from a soldier who acquired his infection in New Guinea in the Aitape-Wewak area (the soldier who provided the "A" strain, already described, came from the same area). This man was suffering from an attack of vivax malaria, and he had 8,000 trophozoites per c mm. in his peripheral blood. P K (500000) received an estimated 160 million trophozoites, which increased the density in his peripheral blood from 0 per c mm to 20 per c mm (immediately after the inoculation).

Trophozoite densities increased to reach 2,300 per c mm on the tenth day and 2,500 per c mm on the 12th day. Ring forms, amoeboid forms, preschizonts, and schizonts were seen, but not a single vivax gametocyte was ever seen. During this period he developed clinical malaria—he was graded (a-c) on day + 9, and (b) on day + 10, but (a) on days 11 to 13. His oral temperature reached 99° F on the seventh day and 102° F on the ninth day, but became normal again by the 12th day after inoculation.

On the 12th day after receiving his inoculum *P. falciparum*, trophozoites (4,000 per c mm) were seen in thick blood films. These trophozoite densities rapidly increased to a maximum of 150,000 per c mm on the 14th day, but during this period the *P. vivax* trophozoite density declined to 500 per c mm, 50 per c mm, 10 per c mm, and 0 per c mm respectively. Treatment commenced on the 14th day and *P. falciparum* densities on the next 4 days were 90,000 per c mm, 50 per c mm, 10 per c mm, and 0 per c mm respectively. Treatment consisted of quinine sulphate (as a mixture) 2.33 grammes a day for 2 days, then quinine sulphate 2.0 grammes for 1 day and on the next 5 days he was given atebirin dihydrochloride 600 mg, 500 mg, 400 mg, 300 mg, and 200 mg respectively. He had a severe attack of falciparum malaria with an oral temperature of 104° F on the 14th day and was graded (e) on the 14th, 15th and 16th days.

Following this mixed attack associated with the finding of *P. falciparum* gametocytes in maximum density of 10 per c mm on the 15th day, he had negative blood films for 68 days, but subsequently occasional trophozoites of *P. vivax* were again found from time to time.

On day + 1,156 (126 days after ending therapy for the mixed infection), he received an intravenous inoculum of 200 *P. vivax* trophozoites (strain "C" from Volunteer 051004, Chart 12). No reaction occurred and no trophozoite wave was demonstrated in his peripheral blood, Chart 13 (d).

On day + 1,166 he was given an intravenous inoculum of 140 million *P. vivax* trophozoites of the "A" strain (from Volunteer 014004) at the same time as three other volunteers already described.

On the day following this injection a trophozoite wave developed reaching a maximum of 530 per c mm on the ninth day, but subsequently diminished to reach 3 to 4 per c mm on the 40th day, when he was discharged without receiving any treatment.

His only constitutional disturbance was a feeling of slight malaise on the ninth day—on oral temperature of over 99.4° F was recorded, and he was not graded less than (a) on any day. His spleen was not palpable during this period.

These four men clearly illustrated that the anti-parasitic mechanism or immunity that they had developed was specific for the "C" strain used for their original infections. They had no evidence of any ability to prevent "A" strain trophozoites from multiplying, in fact the maximal densities exceeded those previously reached with the "C" strain. In this regard it was of some interest to note that, in order of magnitude, the maxima in the "A" strain infections corresponded exactly with those of the "C" strain infections. This phenomenon may have been due to individual variation in the ability to deal with vivax trophozoites.

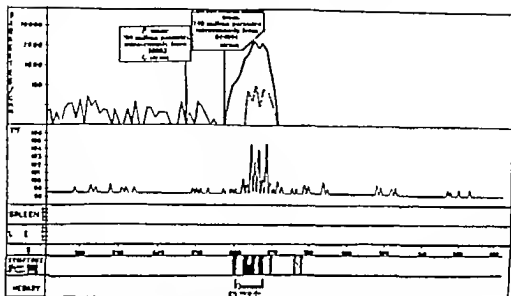


CHART 13 (a) —The demonstration of tolerance without immunity to strain A, *P. vivax* in volunteer (1000029) immune to strain C, *P. vivax*.

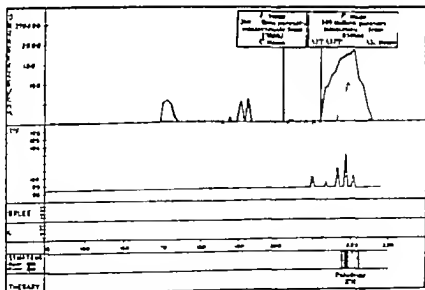


CHART 13 (b) —The demonstration of tolerance without immunity to strain A, *P. vivax* in volunteer (100009) immune to strain C, *P. vivax*.

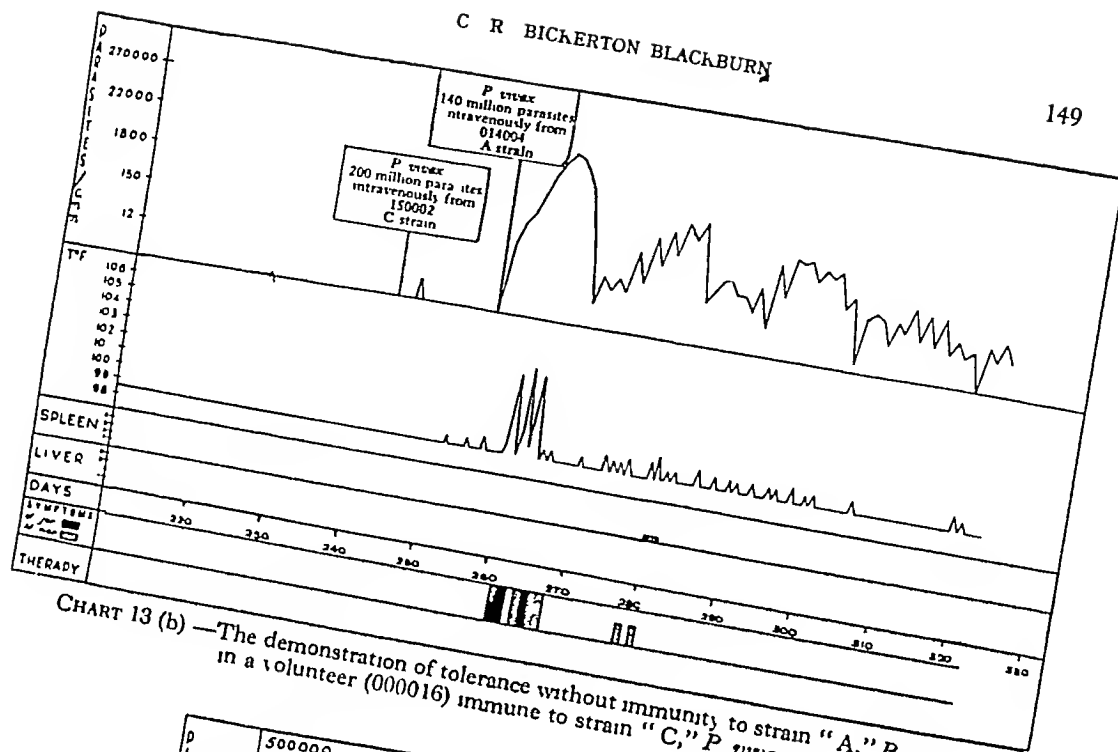


CHART 13 (b) —The demonstration of tolerance without immunity to strain "A," *P. vivax*, in a volunteer (000016) immune to strain "C," *P. vivax*

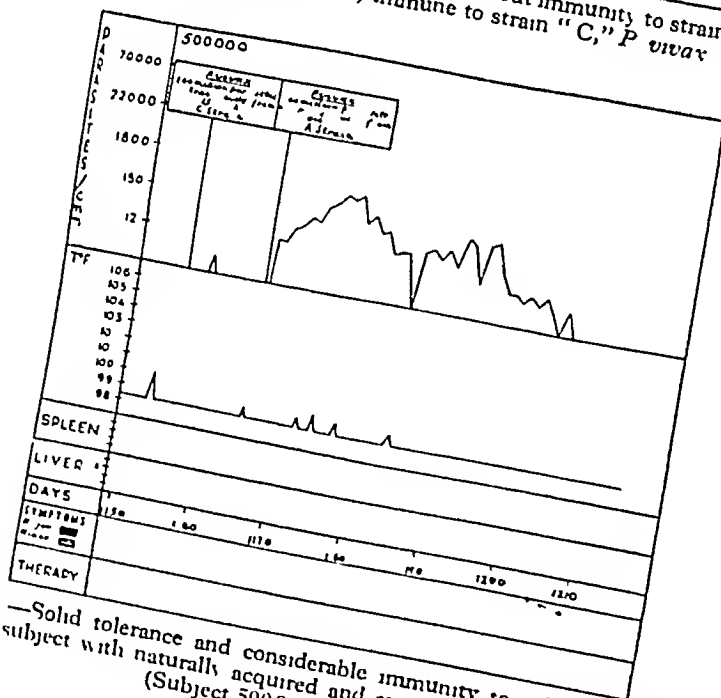


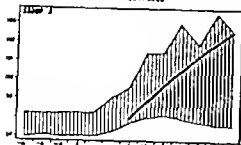
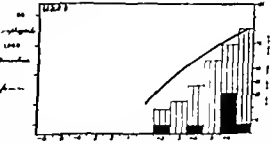
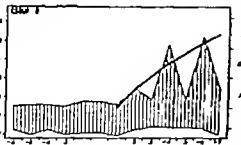
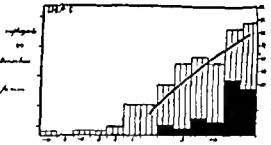
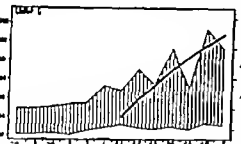
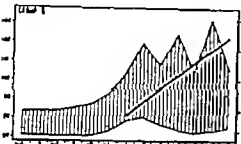
CHART 13 (d) —Solid tolerance and considerable immunity to a heterologous strain of *P. vivax* in a subject with naturally acquired and experimentally induced vivax malaria (Subject 500000, Experiment 8)

In spite of the lack of anti-parasitic immunity all four men showed considerable tolerance to their infections—the lowest parasite densities at which major symptoms and oral temperatures of 100° F or over occurred in the three volunteers were 4,700 per c.mm., and 2,300 per c.mm. respectively whereas in the first attacks the highest density on the development of major symptoms was 1,840 per c.mm. and of temperatures of 100° F or over 280 per c.mm.

P K. (500000) was of interest as he reacted to his first experience of a strain of *P. vivax* from the Aitape-Wewak area (though he was immune to the "C" strain). He had oral temperatures to 102° F when the trophozoite density was between 1,400 and 2,300, though symptoms were minor and his trophozoite wave increased to 2,300 per c.mm. The unexpected development of falciparum malaria (the result of taking blood from a naturally infected soldier with an inapparent *P. falciparum* infection) prevented the adequate observation of his vivax attack. He developed an ordinary attack of falciparum malaria with, perhaps, rather less reaction than might have been expected. His trophozoites reached high numbers—150,000 per c.mm. (3 per cent. of the red blood cells) with concomitant reduction in *P. vivax* densities. He had no appreciable anti-parasitic immunity or tolerance to *P. falciparum*.

Some time after this mixed infection he was again shown to be immune to the "C" strain of *P. vivax* and was then given an intravenous injection of the "A" strain. His reaction was mild as he only had the slightest malaise, but the trophozoites increased in his peripheral blood to reach somewhat higher densities than had been reached in his more recent experiences with the "C" strain. It is possible that both the Aitape Wewak strains were actually the same strain of *P. vivax* as they both came from one small area. If this latter hypothesis is correct, then his degree of tolerance and immunity to the "A" strain was to be expected. By virtue of his tolerance and immunity to the "C" strain, together with his recent experience of the Aitape Wewak strain, he could prevent the trophozoites increasing to densities sufficient to cause symptoms.

This last subject never developed gametocytes of *P. vivax* with any of his infections though the strains were in each instance proved to be able to produce gametocyte waves of normal magnitude in other volunteers and these gametocytes could readily infect mosquitoes. The host appeared to play a major role in the development of gametocytes waves in the peripheral circulation.

FIG. 1. DURATION AND ORAL MAXIMUM OF
TEMPERATURESBAR. PERCENTAGE OF EACH GROUP WITH
MALARIA (Hatched) AND NON-MALARIA (Solid)

1. means of the fitted curves of the daily temperature maxima in the oral and axillary of our group have been represented on these respective charts

CHART 14.—The development of tolerance to vivax malaria. Changes in oral temperatures and in the incidence of symptoms in groups of volunteers with sporadic induced malaria.

calculated by taking the mean of the fitted curves of the logarithms of the daily parasite densities in each man in the group

The striking feature of this chart is the gradual diminution in the degree of reaction, as evidenced by the changes on oral temperature and presence of symptoms, as one passes from Group A to Group E—from primary malaria to malaria in relatively immune subjects

The increased oral temperatures in the different groups show changes in degree, form and time of onset. The total temperature change progressively diminished from Group A to Group E, where the change was minimal, and with this diminution there was a more marked tendency for the temperature records to become tertian rather than quotidian (Eighteen per cent of Group A, but 69 per cent of Group C, had pure tertian attacks)

Though all the groups showed elevation of oral temperatures, the onset of this change was considerably delayed in Groups D and E. In Groups A, B and C, there was a significant rise in oral temperature on the day before trophozoites were demonstrated in thick blood films, in Group D, on the day after they were demonstrated, and in Group E, not until the sixth day of the demonstrable trophozoite wave. These changes in the different groups were not due to slower rates of increase in trophozoite densities. Chart 14 clearly showed that greater densities were required to produce marked elevation of oral temperature in Groups D and E than in Groups A and B.

The change in the lowest daily temperatures was of considerable interest. In primary malaria there was an early and definite rise in the lowest daily temperature, though this did not appear to be a permanent feature of the malaria attack, for it disappeared in the later stages. This change, which was quite evident in the paludrine group (B), supported the previously reported opinion that paludrine acts as a partial causal prophylactic (FAIRLEY *et al*, 1946)—the group behaved like Group A with primary malaria. In Group C, however, this rise in lowest daily temperature was less marked and it was not apparent in Groups D and E. This change was an expression of the early temperature reaction often seen in primary malaria. There may be a continuously elevated temperature during the first few days with no evidence of a daily return to normal, which is so characteristic of the temperature chart in vivax malaria. Chart 4 illustrates this type of temperature record.

This febrile reaction is that referred to as the initial stage by JAMES, NICOL, and SHUTE (1936), and as the initial remittent fever of benign tertian malaria by SWELLENGREBEL and DE BUCK (1938). The latter authors stated that such a type of fever occurred only in primary malaria.

Corresponding closely with these changes in oral temperature, there were changes in the symptoms. There was an increasing delay in the onset of major and minor symptoms, and, when symptoms were present, they were less marked in Groups D and E than in Groups A, B and C. The relations to the trophozoite waves were similar to those already described for the temperature changes—higher parasite densities were required to produce symptoms.

FIG. 10. MEAN MINIMUM AND MEAN MAXIMUM DAILY TEMPERATURES

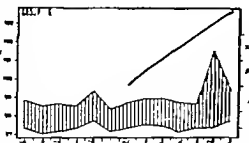
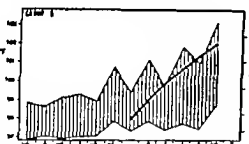
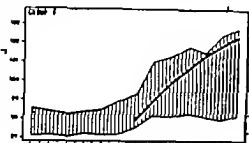
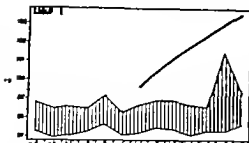
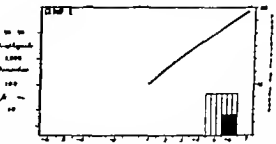
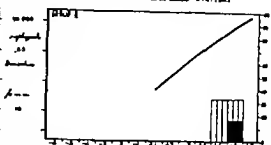


FIG. 11. PERCENTAGE OF EACH GROUP WITH MALARIA BEFORE AND AFTER INDUCED INFECTION



The means of the fixed curves of the daily trophozoite densities. The absolute numbers of no. per. are here superimposed on their respective charts.

FIG. 15.—The development of tolerance to vivax malaria. Changes in oral temperature and in the incidence of symptoms in groups of volunteers with trophozoites or sporozoites induced malaria (see text)

As the infection in Group E (the immune group) resulted from the intravenous inoculation of trophozoites of a different strain from that used for the volunteers in Groups A, B, C and D, Chart 15 was prepared to show that neither the mode of infection nor the strain of parasite was responsible for the differences in Group E. Chart 15 was prepared in exactly the same manner as Chart 14. The groups of volunteers included were as follows —

Group F Trophozoite induced primary vivax malaria. Whole blood containing trophozoites was injected intravenously into volunteers, with no previous exposure to malaria, who were receiving no drug treatment. 14 volunteers.

Group G (corresponded with Group C) Mosquito transmitted secondary vivax malaria. Strain "A" *P vivax*. Volunteers whose primary attacks were suppressed with atabrin dihydrochloride. No previous malaria experience. 5 men.

Group E (as previously described) Immune volunteers infected by the intravenous injection of strain "A" *P vivax* trophozoites. 3 men. Inspection of this chart clearly showed that the reaction in trophozoite induced malaria (Group F) did not differ from the reaction to sporozoite induced malaria (Group A). Further, the reaction to sporozoite induced strain "A" vivax malaria did not differ from the reaction to strain "C" when they were used to infect volunteers receiving atabrin dihydrochloride suppression. In other words, the changes in Group E were not due either to the mode of infection or to the particular strain of *P vivax*.

The differences in reaction of these groups were possibly due to —

- (i) Variation in individual susceptibility—though the groups were small this seemed to be a most unlikely explanation.

- (ii) Persistence of the effects of the various drugs used in some of the groups. This argument was nullified by the findings recorded in Part I. Precisely the same results were obtained in volunteers in whom the question of drug action did not arise.

- (iii) Differences in the behaviour of the trophozoite waves—differences in rates of increase and mean parasite densities. This would mean that changes in tolerance were dependent on the development of more efficient anti-parasitic mechanisms, *i.e.*, on immunity. This did not appear to be so for the trophozoite curves for six of the seven groups were very similar, the only exception was Group E. The equations of the mean fitted curves of the logarithms of the trophozoite densities in the peripheral blood of the seven groups are tabulated below.

TABLE I

REGRESSION OF THE MEAN FITTED CURVES OF THE DAILY TROPHOZOITE DENSITY IN THE PERIPHERAL BLOOD OF GROUPS A TO G.

Groups.	Mean fitted curves (Calculated and expressed in logarithms to the base 10)
A (45 men)	$y = 8.318 + 1.10323 - 0.03831 x^2$
B (37)	$y = 4.8716 + 0.94304 + 0.00173 x^2$
C (29)	$y = 8.2781 + 1.03190 - 0.03401 x^2$
D (8)	$y = 6.7734 + 0.91516 - 0.03431 x^2$
E (3)	$y = 7.1568 + 0.91970 - 0.03504 x^2$
F (21)	$y = 8.4212 + 1.02170 - 0.03003 x^2$
G (5)	$y = 8.5746 + 1.06196 - 0.04437 x^2$

The two groups with the highest mean parasite densities were Groups D and E—the groups with the most tolerance in whom treatment could be delayed longest allowing the mean density to become higher. The trophozoite curves in the tolerant Groups D and E appeared to be a little damped down when compared with those of Groups A and F.

A comparison was made between the mean trophozoite density on the day when the first oral temperature of 100° F. or over was recorded in the eight men making up Group D on whom adequate data were available for both their first and second attacks. The mean trophozoite density in their first attacks was 24 per c.mm. but in the second attack 470 per c.mm. on this day. (Using the logarithms to the base 10 of the parasite densities the difference between the means was 3.0682, and P lay between 0.01 and 0.001—a highly significant difference.) Further in their first attacks oral temperatures of 100° F. or more were first recorded between the first and fourth (average of 2.6) days of the demonstrable trophozoite wave, but in the second attacks between the third and ninth (average of 5.1) days. There was no statistically significant difference between the fitted curves of the trophozoite densities in the first and second attacks. It may be of interest to record that the mean trophozoite density in the peripheral blood of the control Group (A) was 17.5 per c.mm. on the day of the first recorded oral temperature of 100° F. or over.

It seems reasonable to say that the differences observed were not due to differences in rates of increase in trophozoite densities.

(iv) *The previous experience of malaria*—the obvious cause of increased tolerance and immunity. The previous experience refers both to the duration and to the total bulk of the infection (SIVROV, 1939a, 1939b). In Table II the detail of the previous experience of malaria in the various groups has been set out. Accurate figures are given where possible, but in

places estimates have been made which were based on findings in sub-inoculation experiments at Cairns (FAIRLEY *et al* , 1947)

TABLE II
PREVIOUS EXPERIENCE OF VIVAX MALARIA IN GROUPS A TO D

Group	Total duration of infection in days		Previous trophozoite wave				Degree of clinical reaction
			Duration in days		Maximum density		
	Mean	Range	Mean	Range	Meano per c mm	Range per c.mm	
A	12	(9-15)	0		0		0
B	51 6 ¹	(17-107)	(0)		(0)		0
C	66 6 ²	(30-130)	4+		less than 1		±
D	52 1 ³	(42-61)	16	(13-20)	13,000	(4,480-26,300)	++
E	245	(212-257)	227	(180-248)	5,000	(2,320-10,300)	++++
F	8 2	(2-14)	0		0		0
G	50 2 ⁴	(49-61)	4+		less than 1		±

¹ Standard deviation = ± 28

³ Standard deviation = ± 6

² " " = ± 29

⁴ " " = ± 4

This table shows the considerable differences in the duration and bulk of infection in the various groups. Groups B, C, D and G all had approximately the same total duration of infection but there was a great difference between their trophozoite experience which was reflected by the degree of reaction they had experienced.

It seems reasonable to say that the duration of the e e phase corresponds closely with the total duration of infection in sporozoite induced vivax malaria and that trophozoite induced malaria is unassociated with any similar e e phase. In Table III are set out the degrees of tolerance and immunity shown by the various groups in relation to their previous e e and trophozoite experience.

TABLE III
RELATIONSHIP OF TOLERANCE AND IMMUNITY TO PREVIOUS E E AND TROPHOZOITE EXPERIENCE

Group	Previous e e experience (days)	Previous trophozoite experience	Tolerance	Immunity
A	(12) +	—	—	—
B	(52) ++	(—)	(—)	—
C	(67) ++	+	+	—
D	(52) ++	++	++	—
E	(245) ++++	++++	+++	+
F	(0)	—	—	—
G	(56) ++	+	+	+

This table clearly shows that the previous trophozoite experience determined the degree of tolerance to the infection. The e e phase appeared to play no part—a comparison of group A with group F or a comparison of group B with group D clearly showed this.

TABLE I.

EQUATIONS OF THE MEAN FITTED CURVES OF THE DAILY TROPHOZOITE DENSITIES IN THE PERIPHERAL BLOOD OF GROUPS A TO G.

Groups.	Mean fitted curves (Calculated and expressed in logarithms to the base 1).
A (48 men)	$y = 5.2519 + 1.1032x - 0.07631x^2$
B (37)	$y = 4.8716 + 0.8939x + 0.00173x^2$
C (29)	$y = 5.2791 + 1.0319x - 0.03461x^2$
D (8)	$y = 5.7731 + 0.8151x - 0.07451x^2$
E (3)	$y = 7.1568 + 0.8197x - 0.02409x^2$
F (21)	$y = 5.4.13 + 1.0217x - 0.03093x^2$
G (5)	$y = 5.5724 + 1.0619x - 0.07617x^2$

The two groups with the highest mean parasite densities were Groups D and E—the groups with the most tolerance in whom treatment could be delayed longest allowing the mean density to become higher. The trophozoite curves in the tolerant Groups D and E appeared to be a little “damped” down when compared with those of Groups A and F.

A comparison was made between the mean trophozoite density on the day when the first oral temperature of 100° F or over was recorded in the eight men making up Group D on whom adequate data were available for both their first and second attacks. The mean trophozoite density in their first attacks was 24 per c.mm. but in the second attack 470 per c.mm. on this day (Using the logarithms to the base *e* of the parasite densities, the difference between the means was 3.0682, and *P* lay between 0.01 and 0.001—a highly significant difference.) Further in their first attacks oral temperatures of 100° F or more were first recorded between the first and fourth (average of 2.6) days of the demonstrable trophozoite wave but in the second attacks between the third and ninth (average of 5.1) days. There was no statistically significant difference between the fitted curves of the trophozoite densities in the first and second attacks. It may be of interest to record that the mean trophozoite density in the peripheral blood of the control Group (A) was 17.5 per c.mm. on the day of the first recorded oral temperature of 100° F or over.

It seems reasonable to say that the differences observed were not due to differences in rates of increase in trophozoite densities.

(iv) The previous experience of malaria—the obvious cause of increased tolerance and immunity. The previous experience refers both to the duration and to the total bulk of the infection (SINTON, 1939a, 1939b). In Table II the detail of the previous experience of malaria in the various groups has been set out. Accurate figures are given where possible, but in

places estimates have been made which were based on findings in sub-inoculation experiments at Cairns (FAIRLEY *et al*, 1947)

TABLE II
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Group	Total duration of infection in days		Previous trophozoite wave				Degree of clinical reaction
			Duration in days		Maximum density		
	Mean	Range	Mean	Range	Mean per c.mm	Range per c.mm	
A	12	(9-15)	0		0		0
B	51 6 ¹	(17-107)	(0)		(0)		0
C	66 6 ²	(30-130)	4-		less than 1		±
D	52 1 ³	(42-61)	16	(13-20)	13,000	(4 480-20,300)	++
E	245	(212-257)	227	(180-248)	5,660	(2,320-10,300)	++++
F	8 2	(2-14)	0		0		0
G	50 2 ⁴	(40-61)	4+		less than 1		±

¹ Standard deviation = ± 28

³ Standard deviation = ± 6

² , , = ± 20

⁴ " " = ± 4

This table shows the considerable differences in the duration and bulk of infection in the various groups. Groups B, C, D and G all had approximately the same total duration of infection but there was a great difference between their trophozoite experience which was reflected by the degree of reaction they had experienced.

It seems reasonable to say that the duration of the e e phase corresponds closely with the total duration of infection in sporozoite induced vivax malaria and that trophozoite induced malaria is unassociated with any similar e e phase. In Table III are set out the degrees of tolerance and immunity shown by the various groups in relation to their previous e e and trophozoite experience.

TABLE III
RELATIONSHIP OF TOLERANCE AND IMMUNITY TO PREVIOUS E E AND TROPHOZOITE EXPERIENCE

Group	Previous e e experience (days)	Previous trophozoite experience	Tolerance	Immunity
A	(12) +	—	—	—
B	(52) ++	(—)	(—)	—
C	(67) ++	+	+	—
D	(52) ++	++	++	—
E	(246) ++++	++++	+++	+
F	(0)	—	—	—
G	(50) ++	+	+	+

This table clearly shows that the previous trophozoite experience determined the degree of tolerance to the infection. The e e phase appeared to play no part—a comparison of group A with group F or a comparison of group B with group D clearly showed this.

This analysis lends strong support to the findings in Part I of this paper where experiments on individual volunteers were described. Experiment 6 showed the absence of immunity or tolerance in a volunteer who had been infected for some 174 days before he was allowed to have an attack of malaria. His fellows, infected on the same night, were by this time tolerant and immune as a result of considerable trophozoite experience.

DISCUSSION

The data reported here confirm the view that tolerance and immunity develop only after considerable experience of active malaria. The mere presence of malaria infection, as distinct from malaria *disease* resulted in minimal resistance even over a long period of time—indeed one subject had little or no resistance after his infection had been present for over 21 weeks without activity. In contrast, one sharp attack of overt malaria, malaria *disease*, was sufficient to confer a recognizable degree of resistance to subsequent attacks.

The lack of resistance to malaria shown by subjects after they ceased to take suppressive doses of chemotherapeutic agents indicated that the presence of the e.e. phase in their tissues did not result in tolerance or immunity that could be demonstrated. Those subjects who had experienced minor and sub-microscopic, trophozoite waves during their suppressive regimens had more tolerance than those who did not have such waves. The greatest tolerance however developed subsequent to considerable trophozoite experience. Trophozoite induced vivax malaria, for the therapy of paretics, has long been known to confer a degree of tolerance and immunity to subsequent infection with homologous strains, but no satisfactory evidence has been produced to indicate the existence of any e.e. phase in this type of infection. Trophozoite experience was the determining factor in the development of tolerance and this tolerance was followed by immunity—neither tolerance nor immunity developed in the absence of trophozoite experience.

The development of demonstrable immunity took considerably longer than did the development of tolerance. Several subjects appeared to have less ability to deal with their increasing trophozoite densities during their first secondary attacks than during their primary attacks. In this regard it has long been recognized that trophozoite densities in relapses tend to reach higher levels than in primary attacks—this is not solely dependent on the fact that more time is available for high densities to be reached in secondary attacks (owing to the development of some tolerance). With this passage of time the developing immunity becomes more efficient—in the early stages a given dose of trophozoites given intravenously would "break down" the immune mechanisms, but the same dose would completely fail to do so at a later date. Subjects with malaria could develop a very solid immunity that could not be

disturbed by the injection of huge numbers of trophozoites of the homologous strain. Certain stresses also failed to disturb this type of immunity.

Tolerance was not strictly strain specific. Considerable tolerance was developed against one heterologous strain used in the experiments. In contrast to this, immunity was strictly strain specific as no immunity could be demonstrated against a heterologous strain of *P. vivax*. The three volunteers used to demonstrate the lack of cross immunity actually developed their highest trophozoite densities when infected with the heterologous strain.

Subjects who had developed solid tolerance and immunity to their infections constantly had trophozoites in their peripheral circulations but the densities rarely exceeded 1 per cmm. over several months of observation. In spite of this, gametocytes were never seen during the period of immunity and mosquitoes fed on these subjects did not become infected. The low densities of trophozoite caused no constitutional disturbances whatever, the subjects were positively normal as distinct from being symptom free. They had normal red blood cell and white blood cell counts, haemoglobin concentrations at least of their pre-infection levels, their weight was maintained at their normal range, their spleens were impalpable and they were perfectly fit. No subjects ever developed any syndrome that could have been called "low fever."

The excellent health of tolerant and immune individuals, as contrasted with the absence of resistance in individuals whose malaria had been suppressed with various chemotherapeutic agents, naturally raises the question of the optimum form of curative, palliative and suppressive treatment.

Curative treatment for sporozoite induced *vivax* malaria cannot be described as more than on the horizon. Guarantees cannot yet be given. There are several excellent drugs available for palliative therapy—the clinical cure of the attack without eradication of the infection. Continued suppression can readily and safely be attained even under adverse conditions. The following forms of treatment are available for use in suitable conditions.

(a) Intensive treatment as soon as the diagnosis is established in the hope that radical cure will be obtained.

(b) Delayed treatment—allowing the patient to have considerable trophozoite experience—so that he may acquire some tolerance and immunity.

(c) Complete and long-continued suppression of trophozoites with the idea that the infection may "die out" with the passage of time.

(d) Intermittent or partial suppression for such a period of time that will allow the patient to develop tolerance and immunity from his frequent, minor trophozoite experience. When this tolerance and immunity have developed the suppression would be ended with a relatively complete natural disinfection. The observations recorded in this paper do not supply a definitive answer to the question of which type of treatment is best.

In deciding the desirability, or undesirability, of a type of suppression for a given individual the following factors must be considered as a background of knowledge of when and how resistance against malaria develops.

The parasite: the strain of *P. vivax* causing the infection is most important. One must know the usual duration of the latent period between primary attack and first secondary attack. (Some European strains have latent periods of 8 months, but the S.W.P.A. strains have very short latent periods.) The natural duration of the *tert.* phase and the usual duration of the clinical attacks are both important, the infection may characteristically die out in 1, 2, 3 or more years.

The individual: his general health must be considered—obviously it is most undesirable for a patient with active tuberculosis to have even a single attack.

The geographical conditions: good advice in a non-malarial area may be bad advice in an hyperendemic area. If a person is moving from place to place he may encounter multiple heterologous strains and species, and will be unlikely to develop adequate immunity.

Time: obviously different advice would be given to a person taking up permanent residence in a hyperendemic area from that given to one visiting the same area for a month or two.

The drugs: the availability of curative drugs and the possible effects of prolonged administration of suppressive drugs must both be taken into account.

It has been suggested that it would be better to withhold therapy in acute vivax malaria to let the subject develop tolerance and immunity as fast as possible. This is obviously unsound advice in hyperendemic areas where there may be mixed infections which are not apparent at first sight. In non-malarial areas it is not recommended as a routine as there are now drugs available which do produce a proportion of radical cures (plasmoquine, pentamquine, paludrine) and there are dangers to the patient if treatment is withheld. Two patients developed subcapsular splenic haemorrhages and one developed agranulocytic angina during the acute phase of untreated vivax malaria at Cairns—both the subjects were healthy young men prior to exposure to these infections. In certain individuals, however, some of the factors discussed in the previous paragraph may make the withholding of treatment desirable so that tolerance and immunity are developed.

With the drugs available at the present time each person or group of persons must be treated according to the circumstances—any of the methods of treatment mentioned may be the optimum at one time and place but far from it at another. The patient and his circumstances, the host-parasite relationships, and the drugs available determine the treatment to be used.

The excellent health of the volunteers who were tolerant and immune to their continued slight parasitaemia clearly placed them in the category of malaria infection without malaria disease. The failure to disturb their equilibrium by severe chilling and alcoholic excess, together with the complete absence of fever or splenomegaly made a diagnosis of chronic malaria untenable. One should be sceptical about a diagnosis of chronic malaria given to minor illnesses characterized by malaise, headache and perhaps slight chilliness and fever simply because the patient had vivax malaria some time in the past. This especially applies to non-malarial areas.

CONCLUSIONS

- 1 Tolerance to the effects of *vivax* malarial developed before an enhanced ability to deal with the invading parasite
- 2 Tolerance and immunity developed in response to *P vivax* trophozoite activity rather than to activity of exo-erythrocytic forms, the degree of tolerance and immunity depended on the bulk of the trophozoite experience of the individual
- 3 Volunteers who had become tolerant to one strain of *P vivax* showed definite tolerance to trophozoites of another strain of *P vivax* on their first experience of the second strain Tolerance to *P vivax* is not necessarily strain specific
- 4 Subjects who had become immune to one strain of *P vivax* did not exhibit any enhancement of their anti-parasitic mechanism when infected with trophozoites of another strain Anti-parasitic immunity may be strain specific

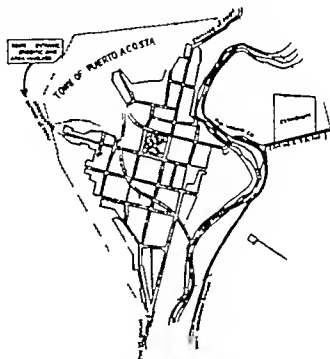
5 Subjects who had developed complete tolerance and solid immunity to their trophozoites were perfectly fit, had palpable spleens, and had normal or super-normal haemoglobin concentrations though trophozoites could be found in their circulating blood whenever adequate examinations were made Attempts to induce malaria attacks by severe chilling and alcoholic excess were unsuccessful

6 Gametocytes were never seen in the peripheral blood of subjects who had developed complete tolerance and immunity to their infection Mosquitoes were not infected by the blood of these immune subjects

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afforded by an epidemic of typhus which occurred in the province of Camacho, Department of La Paz, Bolivia, during late 1947. The province of Camacho, with a population of approximately 120 000 90 per cent. of whom are pure Aymara, lies 203 km. north of the capital La Paz, on the eastern shore of Lake Titicaca and borders Peru on the north. Puerto Acosta (population 1,200) is the seat of the provincial government. The climate is rigorous, the altitude being approximately 14,000 feet above sea level and surrounding snow-capped mountains cause the temperature to remain quite low.



Plan of the town of Puerto Acosta. Area ravaged by typhus epidemic is shaded.

Originating across the border in Peru during October 1947 the epidemic spread south and east into Bolivia, reaching Puerto Acosta in mid November. It persisted in Puerto Acosta until about the 15th December when the activities of Servicio Corporativo Inter Americano de Salud Pública checked its progress by means of immunizations and a vigorous DDT campaign. During the two months of the epidemic, deaths from typhus amounted to at least 60, the majority occurring in the bordering villages of the canton of Conima in Peru. Later the epidemic spread to other provinces, where this study was continued.

A preliminary report by PARKE and KNAUTH (1948) of the first cases treated has already appeared.

TABLE

Case	Age and sex	Weil-Felix	Temp ° C	Pulse	Dose (gramme)			Return to normal		Symptoms relieved
					Daily		Total	Temp	Pulse	
					Oral	I V				
								Hours	Hours	Hours
1	15 F	1 1,200	40.9	140	1.0	—	2.5	48	54	60
2	16 F	1 1,200	39.7	130	1.0	—	2.5	48	48	60
3	12 F	1 1,400	40.0	140	—	0.4	1.2	54	48	72
4	14 M	1 1,200	41.0	140	—	0.5	1.25	48	36	54
5	32 F	1 1,200	39.0	120	2.0	—	4.0	48	48	48
6	18 M	1 1,400	39.5	130	1.5	—	3.0	42	46	36
7	17 M	1 1,200	40.0	140	—	0.8	2.8	24	24	42
8	38 F	1 1,200	40.2	140	1.0	0.8	4.2	32	36	48
9	18 M	1 400	38.9	120	2.0	—	3.5	24	30	48
10	45 M	1 600	Sub-normal	130	1.0	1.0	5.8	48	30	72
11	48 F	1 1,200		140	1.5	1.0	4.0	38	38	52
12	38 F	1 600		120	2.0	0.8	3.9	24	30	60
13	30 M	1 1,200	39.4	120	3.0	—	3.8	24	24	48
14	25 M	1 1,200	40.3	140	3.5	—	3.9	24	24	48
15	29 F	1 1,200	40.1	140	1.5	1.2	4.0	24	24	48
16	52 M	1 1,200	39.6	120	2.0	0.6	4.0	24	24	48
17	22 M	1 1,200	39.7	140	1.5	—	3.0	24	24	36
18	48 M	1 1,200	40.2	140	1.5	—	3.6	36	42	54
19	17 F	1 600	39.7	120	1.5	—	3.0	26	30	42
20	43 M	1 1,200	39.5	120	1.5	—	3.0	24	24	48
21	54 M	1 600	39.7	140	1.5	—	3.0	28	36	48
22	42 F	1 400	40.6	140	3.0	0.3	8.8	44	48	72

The Table presents the 22 cases of epidemic typhus treated with chloromycetin. The results in tabulated form show the rapid recovery of the patients following treatment. In this list Cases No 8, 11, 14 and 19 presented signs and symptoms of probable death. Cases No 10, 15, 16, 18 and 22 gave evidence of certain death.

CONTROLS

For controls we studied 50 untreated cases of typhus occurring in the same epidemic, with the following results:

Weil-Felix at dilutions of 1 600 to 1 1,400, average 1 1,200 (Readings done on 37 cases.)

Number of deaths 14, or 28 per cent.

Deaths occurred on 11th to 20th day of disease, average 14 days (In six cases the time was not exact.)

Patients recovering without treatment with chloromycetin entered convalescence on 12th to 26th day of disease—average, 18 days.

Space does not permit the publication of the extensive records of each case reported, and since the symptoms and progress are quite similar only three brief case records are herewith presented.

CASE No. 8. Basilia de Zalica. General Hospital, La Paz. Female. Age 33 years. Colour white. Weil-Felix reaction positive in dilution of 1:1,200.

17th December 1947. This patient exhibited all the signs and symptoms of grave infection of epidemic typhus. Onset 6 days ago was violent with chills, vomiting, intense headache and backache. The patient had insomnia and was stuporous. On examination clear generalized exanthema was found accompanied by conjunctivitis, partial paralysis of tongue, coughing and dyspnoea.

R.B.C., 4,200,000 W.B.C., 4,110 Hb., 39 per cent. Urine albumin, trace casts, negative.

The temperature 40.2° C. Pulse 140. Prognosis, grave.

18th December 1947. In consultation the medical staff agreed that the patient had lost ground during the night. The symptoms and physical findings were more pronounced. There was profound stupor interspersed with delirium. Treatment with chloromycetin was begun at once.

Treatment.

Hour	Chloromycetin, Gramme.	Temperature	Pulse.
11.30 a.m.	0.2 intravenously	40.6° C.	140
2.30 p.m.	0.2 intravenously	39.5° C.	120
5.25 p.m.	0.2 intravenously	38.6° C.	90
	0.5 orally		
9.30 p.m.	0.2 intravenously	38.0° C.	120
	0.5 orally		

At the time of the 9.30 p.m. dose there was noted obvious improvement in the patient's condition, and she was found asleep soon afterwards.

19th December 1947. Morning: The cephalgia, rachalgia and polydipsia were less intense than on the previous day. Improvement was noted in the general appearance of the patient.

Treatment.

Hour	Chloromycetin, Gramme.	Temperature	Pulse.
10.30 a.m.	0.2 intravenously	37.5° C.	100
	0.5 orally		
9.30 p.m.	0.2 intravenously	36.6° C.	80
	0.5 orally		

During the afternoon the improvement became more pronounced. All headache and backache disappeared. The patient sat up to eat her evening meal, and enjoyed 'good night' sleep.

R.B.C., 4,080,000 W.B.C. 4,000 Hb. 37 per cent. Urine albumin, trace; casts, negative.

20th December 1947. The patient felt well, all symptoms had subsided and the petechiae were almost gone.

Treatment.

Hour	Chloromycetin, Gramme.	Temperature	Pulse
10.30 a.m.	0.5 orally	36.5° C.	80
5.30 p.m.	0.5 orally	36.6° C.	80

Recovery was so far advanced that treatment was suspended, and the patient allowed up Observation during the succeeding 10 days failed to show any relapse of fever or symptoms

Forty-eight hours after treatment R B C , 4,000,000 , W B C , 4,200 , Hb , 35 per cent Urine albumin, trace , casts, negative

Seventy-two hours after treatment R B C , 4,070,000 , W B C , 4,500 , Hb , 35 per cent Urine albumin, trace , casts, negative

Six days after treatment R B C , 5,700,000 , W B C , 6,100 , Hb , 49 per cent

Total chloromycetin given 4.2 grammes

CASE No 10 Gregorio Zalles General Hospital, La Paz Age 45 years Race, Aymara Early Weil-Felix reaction was positive in dilution of 1:600

The length of time this patient had been ill is not certain, but somewhere between 12 days and 3 weeks During the previous 5 days that the patient had been in the hospital he had received three daily doses of tartar emetic, 3 c c each Also intravenous glucose solution, cardiac tonics, and general symptomatic therapy His condition grew steadily worse from the first violent onset of the disease

Blood specimen lost Urine albumin, + + , casts, present, granular

18th December, 1947 The patient was presented to the authors for treatment with chloromycetin On examination a middle-aged male was found in a coma, who had not spoken or taken food or water for three days He was emaciated, his eyes sunken and lustreless The mouth was foul with sordes and the mucous membrane contained many lesions The temperature had fallen to sub-normal

The heart was weak and irregular, the pulse, faint and thready, averaging about 120 to 140 Respiration was of Cheyne-Stokes type The patient appeared to be *in extremis*, and this impression was verified when it was found that the death certificate had been completed, except for date, and the burial arranged

Treatment with chloromycetin was begun with little hope of success, due to the advanced state of the disease

Treatment

Hour	Chloromycetin Gramme	Temperature	Pulse
11 40 a.m.	0.2 intravenously	35.0° C	120 ±
2 30 p.m.	0.2 intravenously	35.2° C	110 ±
6 45 p.m.	0.2 intravenously	35.1° C	110 ±
10 10 p.m.	0.2 intravenously	35.0° C	100

Forty minutes after the first dose the patient aroused and spoke for the first time in 3 days He asked for and drank a glass of water He remained in a stuporous condition throughout the night, the only improvement noticed was the strengthening of the pulse

Blood specimen lost

19th December, 1947 In general the patient seemed to be about the same except pulse had returned to normal, it was regular and of fair volume and strength

Treatment

Hour	Chloromycetin Gramme	Temperature	Pulse
9 10 a.m.	0.2 intravenously	35.1° C	80
1 30 p.m.	0.2 intravenously	35.0° C	80
4 10 p.m.	0.2 intravenously	35.7° C	80
7 30 p.m.	0.2 intravenously	35.2° C	80
10 20 p.m.	0.2 intravenously	35.0° C	80
	1.0 orally		

As night approached the patient began talking He moved his tongue freely, and called repeatedly for food and water which was furnished

The prognosis has changed rapidly for the better

20th December, 1947 Cardiac tones and pulse were good this morning The patient is fairly alert, and drinks water frequently, no doubt due to the extreme dehydration

Treatment.

Hour	Chloromycetin. Gramme.	Temperature.	Pulse.
9.20 a.m.	0.2 intravenously 0.5 orally	36.8° C.	90
8.30 p.m.	0.2 intravenously	36.6° C.	80
11.45 p.m.	0. intravenously 0.5 orally	36.6° C.	80

R.B.C. 2,700,000 W.B.C., 3 100; Hb., 29 per cent. Urine albumin, ++ casts present, granular

14th December 1947 The patient passed good night and awoke free of symptoms except for slight glossitis.

Treatment.

Hour	Chloromycetin. Gramme.	Temperature	Pulse.
8 10 a.m.	0. intravenously 0.5 orally	38.2 C.	100
11.30 p.m.	0.2 intravenously	36.6° C.	80

22nd December 1947 Beginning on this day the patient was allowed to sit up and feed himself. He appeared to be convalescing rapidly.

Chloromycetin, 0.5 gramme was given orally in the forenoon as the last specific medication. Liver extract and polys vitamins were prescribed to combat the anaemia and malnutrition, caused by the disease.

The patient was kept in the hospital for treatment of severe decubitus. His return to normal health was steady and uneventful.

Total chloromycetin taken, 5.60 gramme.

Ten days after treatment was started R.B.C., 3 900 000 W.B.C., 4 700; Hb., 35 per cent. Urine Albumin, trace casts, negative.

CASE No. 17 Daniel Anemia. Student of medicine Age 23 years. Race white Weil-Felix reaction in dilution, 1:120

21st February 1948. The present illness began 8 days ago with sudden onset of severe intense headache and backache and nausea with mucus vomiting with intense thirst. On examination there were petechiae over the chest, abdomen, and extremities marked glossitis, and conjunctivitis, and light cough with severe dyspnoea. The patient was suffering from periods of delirium and stupor.

Treatment.

Hour	Chloromycetin. Gramme.	Temperature.	Pulse.
11.20 m.	1.5 orally	39.7° C.	140
2.00 p.m.		39.1 C.	120
5 10 p.m.		38.8° C.	120
8.30 p.m.		37.9 C.	110
11 15 p.m.		37.6° C.	110

Treatment was begun with single daily oral dose of 1.5 gramme chloromycetin.

22nd February 1948 The stupor had diminished along with the headache and backache. Delirium was no longer present.

Treatment

Hour	Chloromycetin. Gramme.	Temperature.	Pulse.
9.30 a.m.	1.5 orally	37.4° C.	100
12.10 p.m.		36.7° C.	80
3.20 p.m.		36.4° C.	82
6.30 p.m.		36.6° C.	80
9 10 p.m.		36.8° C.	80
11 15 p.m.		36.4 C.	80



Case 10 Typhus patient with grave prognosis



One week after completion of treatment

23rd February, 1948 The temperature and pulse were normal and remained so during the following days of observation Symptoms of the previous days had disappeared and the patient was feeling well He left the hospital 25th February, and resumed his assignments the next day

Blood Studies

Complete blood counts were done on all patients and five normal controls before, during and after treatment with chloromycetin No consistent change was observed in either white or red cells, and the changes which did occur either fell within the possibility of error or could be explained by improvement of the circulatory blood following the termination of fever

DISCUSSION

Chloromycetin was supplied in two forms For intravenous use, 0.1 gramme per c.c. was dissolved in propylene glycol and finished in rubber-capped vials containing 10 c.c. Tablets for oral medication each contained 0.1 gramme of chloromycetin

The results following slow intravenous injection were rapid Three hours after the first injection, the headache and backache showed improvement and vision was often normal

The solvent must be considered as a possible complicating factor in these results, but, in the authors' opinion, it is of minor importance

Oral dosage was equally effective but required 8 to 12 hours longer for results to appear Later, it was found that many of the tablets were excessively compressed and required several hours to disintegrate

For convenience, the dosage regime adopted toward the end of the study was 1.5 gramme daily as a single oral dose for 2 or 3 days This treatment gave uniformly satisfactory results

Patients treated during this study varied in age from 12 to 52 years There were both Indians and white patients included in the group studied

No toxic reactions or signs of intolerance were observed in the dose range used The blood count did not vary outside the limits of error for field estimation Five normal controls who took the drug for 3 days helped to confirm this observation

The rapid beneficial effect on the heart actions suggests that chloromycetin has the additional virtue of being a myocardial tonic

CONCLUSION

1 Chloromycetin has been a safe antibiotic for intravenous use in the dosage used Indications are that the oral dose may be increased with safety over the intravenous amounts employed

2. The favourable effects of treating typhus (epidemic) with chloromycetin appear rapidly and the patient usually enters convalescence within 3 days.
3. Chloromycetin is effective either parenterally or orally

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MITE TYPHUS FEVER IN ASSAM AND BURMA, 1944-1946 *

BY

P H A WILLCOX, M A, M D, M R C P (LOND), Major (Hon), R A M C †
Late Medical Specialist India Command and S E A C

Until 1935 little was understood about the varieties of typhus fever which were known to exist. MEGAW had studied cases in Central India supposed to be caused by ticks to which patients had often been exposed. There was no local sore and the Weil Felix reaction was negative to *Proteus* OX19.

In 1935, MACNAMARA reported cases occurring in the Simla Hills. This report was followed by further studies by BUSH and COVELL (1936). These writers discriminated between the two varieties of typhus, the OX19 and the OXK type, the urban and rural, the mid-winter and the late summer type. BOYD (1935) described 43 cases in military personnel in India. These cases were those from which sera was sent to a central laboratory for Weil Felix reactions. The cases had originated in Bengal, Assam, Punjab, Central India and the Madras Presidency. MAITRA and GUPTA (1936) reported positive OXK agglutination in cases from widely separated districts in upper and lower Burma. WOODHEAD and DUTTA later (1941) reported positive agglutination in eight out of 203 specimens of sera from Assam. The disease was thus endemic in the areas mentioned, and probably more widely than was recognized. But the low incidence of cases attracted little attention and accounted for the minor interest attached to the disease before the entry of Japan into the world war.

* Some introductory matter has had to be omitted for reasons of space —ED

† I wish to thank Maj-General Sir A. G. BIGGAM, Sir ARTHUR MACNALT and Dr CHARLES WILCOCKS for help and advice. To Lieut-Colonel J. R. AUDY, R A M C, I am indebted for much information and criticism, and to Medical Officers, Sisters and Nursing Orderlies for their loyal co-operation in the treatment and nursing of cases.

The Burma campaign and the fighting on the Assam Burma border caused an alarming increase in incidence reaching a climax in the autumn of 1944. In all approximately 5000 cases with 350 deaths occurred in 1944 and the disease was the most serious medical problem, both from the point of view of the mortality and morbidity to troops and the embarrassment of medical organisation.

Captured Japanese reports dated September 1944 revealed a high incidence among their troops stationed along the course of the Chindwin river in North Burma and in the neighbourhood of Mandalay. The incidence of cases is not recorded.

The serious outbreak of cases among our troops in 1944 was the biggest outbreak ever recorded in history and a good example of an endemic disease suddenly become epidemic in character.

In previous years fighting in Burma automatically subsided for the 5 months beginning in June when monsoon rain turns roads into quagmires and the jungle into steaming swamp. In 1944 it was decided to launch offensives from Imphal in two southward directions simultaneously namely on the roads to Tiddim and Tamu respectively. The troops on the central section of the front were scattered throughout the hilly jungle country at a time of year most favourable for the development of mites. In many places the undergrowth consisted of secondary vegetation which had arisen on land previously under cultivation by the natives. Indeed, it was often the most dangerous types of scrub on which units were encamped. Troops were patrolling affected country and lying down to rest. Reinforcements were exposed in the same way fresh units often took over sites previously used by other units and usually infested with rats. It is thus easy to see that the army was exposed to the bite of larval mites in the most extreme degree, and infection which had hitherto been sporadic became epidemic. The disease tended to arise among troops operating in certain islands of country where suitable vegetation existed for the breeding of mites, though in the changing circumstances of the campaign it was impossible for medical officers in hospitals to ascertain from patients the exact sites of origin. The Typhus Research team did valuable work in tracing islands of origin, in investigating local flora and fauna, and in advising officers of probable infested sites.

From the point of view of the campaign, the late summer and autumn of 1943 and 1944 were the most serious periods. It had been known that typhus fever had occurred during the Burma retreat of 1943 accounting for some deaths, though no records are available. In 1942, 20 cases had occurred in the environs of Calcutta, and 33 cases at Ranchi, Bihar in 1943. There were only 20 cases in the Imphal area from January to September of that year. On 11th October 1943, two companies of a British regiment patrolled a hill about 2 miles long, the jungle of which was replaced by grass and young palms. In all, 121 cases occurred in that unit commencing on 20th

October (TATTERSAL, 1945) This outbreak, which was known—from study of the incubation periods—to have originated from this area near Moreh, illustrates the importance of "islands" of infection in the jungle

The siege of Imphal was raised in May, 1944. In July and August the disease incidence increased as the troops advanced southwards along the Imphal-Tiddim and the Imphal-Tamu roads.

The task of keeping the Japanese in retreat to Tiddim fell to the 5th Indian Division, while the 11th East African Division took up the chase of the enemy along the Kabaw valley in torrential monsoon rain. This rapid and famous advance will ever remain a memorable feature of the campaign to anyone who was privileged to serve there. But it was carried out successfully at the cost of many casualties. Nearly 1,400 cases occurred in October and November alone.

The writer was privileged to see most of the sick casualties of this division who were evacuated down the bumpy Tamu road to Palel. Many had travelled up to 100 miles and arrived in hospital after 2 days of uncomfortable travelling, often in a collapsed condition. The typhus cases amongst these casualties form the bulk of the cases discussed in this paper, most were East Africans, but there were some British and Indian cases in addition.

The objectives at Kalembo and Kallewa were reached in December, 1944, by which time there was a marked fall in the number of cases. This was attributable both to the cessation of hostilities in the affected areas and to seasonal change. From a study of the relatively high mortality figures, the severity of infection in those patients that recovered and the severe debility following the illness, it is clear that this disease was more serious from a military point of view than malaria or dysentery, the incidence of which was anticipated. These cases of typhus all required most careful nursing for long periods which could not always be provided in the forward medical units. The evacuation to the base by air was not possible in many of the most serious cases and those first seen at the end of the first week of the disease.

CLINICAL ASPECTS

This paper is a study of 493 cases of mite typhus fever, all of which came under the care of the writer between January, 1944, and April, 1946. They were seen in different parts of Assam and Burma, and may be grouped as follows —

Period	Site	British	African	Indian	Japanese	Total
Jan -March, 1944	Tiddim Rd	4	0	0	0	4
July-Dec., 1944	Imphal and Palel	30	361	79	0	470
1945-6	Rangoon and North Burma	0	0	7	0	7
1945	Rangoon	0	0	0	6	6
		40	361	86	6	493

The series includes a large number of East African troops who succumbed to the disease during their advance from Imphal to Tarnu and Kalewa in 1944. The study can be said to describe the disease as it occurred in British, African and Indian subjects, from a clinical point of view. No elaborate pathological study of the disease was possible in the circumstances prevailing in the campaign. The standards of diagnosis and treatment are discussed, and the clinical findings compared with those of other authors. Patients were treated in a 400-bedded Indian hospital to which were attached East African sections in 1944 and in 1945-46 13 cases were treated in a small Indian hospital working under static conditions.

Incubation Period. Authorities differ with regard to the incubation period. The reason for this is that the patient is usually unaware of the mite bite and rarely can remember the time of its occurrence. During the course of this campaign the bulk of the troops had been in possibly infected areas of country for too long periods for estimation of the incubation periods.

TATTERALL (1945), in his account of an outbreak among troops of British regiment near Moreh, established the incubation period of 9 to 17 days for all cases. MITCHELL (1945) records 7 to 21 days, LAWTERWHITE and SAVOOR (1940) 11 to 21 days. Owing to language difficulties, it could not be estimated in the present series, except among the British and Indians, from whom no useful information was forthcoming.

Onset. Fever normally begins suddenly though the rise in temperature is steplike, in the first 4 days. The height of the fever is seldom reached before the third day. The fever is preceded by malaise for 2 to 3 days. A proportion of cases the onset of fever is gradual. One such case was seen in which the temperature rose no higher than 100.4°F in the first 4 days. The presence of few papular spots on the chest and abdomen raised the possibility of smallpox rash, but the patient was found to have shallow ulcer in the right axilla—an obvious sign of the primary lesion from mite bite. He subsequently had typical attack with widespread rash and fever for 18 days, followed by lysis.

With this exception, the majority of cases were not seen till the third or fourth day the onset could not therefore be studied in detail.

Ragons are few or absent and are only seen when the temperature rises rapidly.

Vomiting is rarely seen in typhus. Its presence suggests some other condition or concurrent disease.

SPECIAL FEATURES.

Headache is universally the most frequent complaint and is usually frontal. Occipital pain is associated with palpably enlarged and tender glands in the occipital region or in the posterior triangles of the neck.

Neck rigidity associated with other meningeal signs, was encountered in seven cases. In all these cases lumbar puncture was performed in order to investigate the pressure and cell count of the cerebrospinal fluid. In all cases the C S F was quite clear.

The findings are shown in Table I.

Backache and pains in the joints are commonly troublesome.

Mental Symptoms. Drowsiness or apathy was almost always present. This was of such degree as to cause delayed cerebration, lack of attention and disinclination to feed, drink or speak.

TABLE I

	Date of onset	Day of disease Lumbar puncture performed	CSF		Outcome
			mm water press	emm cell count	
1	8.8.44				
2	26.9.44	20th			Death 22nd day
3	14.11.44	10th	110	40 LA	12th
4	5.11.44	14th	175	120 "	15th
5	23.11.44	22nd	100	50 "	10th
6	17.10.44	20th	186	42	21st
7	7.8.45	20th	120	10	72nd
		0th	110	20	(lung abscess)
			220	160	Recovery

It will be seen that meningeal signs are of grave import, and in this series were seen particularly as a feature of the terminal phase of the illness. If present at an early stage of the disease, they are an indication of the intensity of cerebral toxæmia. Where the pressure of the CSF is markedly raised, benefit may result from the careful withdrawal of a few c.c. of CSF.

After recovery retrograde amnesia for this period is commonly found in severe cases. For example, the severity of the headache is forgotten and mental symptoms are forgotten in the same way that the pains of labour are forgotten in the puerperium.

Deafness Though only present in almost one-third of the cases, this is a valuable sign of mite typhus, though of course deafness also may occur in louse-borne typhus fever. The exact nature of the lesion is unknown. Recovery is rapid and normally complete.

TABLE II

Total cases investigated	Number deaf	Per- centage deaf	Onset of deafness			Mortality of deaf cases
			Average	Maximum	Minimum	
400	98	24.5	14th	27	7th	11 = 2.3%

At the beginning of the second week mental symptoms become more pronounced in severe cases. Drowsiness is usually more intense, but some of the following features may appear —

Restlessness, excessive talkativeness, delusions, delirium with muttering, tremors of lips and tongue, picking at the bed clothes, subultus tendinus, coma and convulsions, incontinence of urine and faeces.

Restlessness is sometimes so accentuated as to render the patient maniacal and violent. In one case the patient left his bed in the middle of the night and sustained fall on the ground outside his ward. Six days later he died, and at postmortem rupture of the spleen was found, although this was not considered the cause of death.

Hiccough. This symptom was present in six cases, three of which were fatal. The three patients who recovered were seriously ill. In one case the duration of hiccough was 8 days. In two cases in which a postmortem was performed, basal adhesions were found indicating diaphragmatic pleurisy.

Tendon Reflexes. The deep reflexes were examined in 20 consecutive cases in the second week. In all these they were not elicited or were extremely weak in response. The return of normal responses was slow and delayed, probably as the result of wasting of muscles. WILLCOX, W. H. (1923), in describing typhus exanthematicus, states that the tendon reflexes are almost invariably abolished. The importance of the sign is in the distinction from typhoid and paratyphoid fever in which the responses are lost only in severe cases.

Respiratory System.

Cough is almost invariably present. It is dry and distressing at night. Sputum is scanty and consists of mucus except where broncho-pneumonia is present from secondary infection, in which case the sputum may be blood-stained or purulent.

In the first week the respirations are rapid and shallow. This frequently leads to the suspicion of pneumonia, but the chest signs are not found to correspond with the degree of increase in the rate of breathing. On examination, dry rales and rhonchi are audible throughout the chest, both on inspiration and expiration. These signs are often mistaken for those of asthma and the distinction of typhus from malaria in an asthmatic subject may be difficult. In the second week signs of broncho-pneumonia may be found usually at the bases posteriorly. Dullness, moist rales and absent breath sounds, though bronchial breathing may be present. Often pleuritic pain is a prominent symptom and friction may be audible over any area of the chest. From postmortem experience, it is found that pleurisy is a very common finding and it is probably present in all cases of typhus in which respiratory complications are present.

Pharyngitis was found in a number of cases in which the throat was repeatedly examined. The throat is red and injected, the pharyngeal wall being injected and oedematous. Ulceration is never seen. The condition was seen, particularly in those cases in which marked cervical adenitis was present. TATTERSALL (1945) records these changes in as many as 34 per cent. of his cases.

Cardiovascular System.

In proportion to the degree of fever the heart rate is not very rapid in the first week. During the persistence of the fever in the second week, the rate

becomes more rapid. Particularly is this likely where the temperature is excessively high. The rapidity of the heart at the end of the second week is of importance in prognosis. Concurrently with the increase in the rate of the heart, there is a tendency for the blood pressure to fall at this period, and if the fall of the systolic pressure to below 80 mm Hg occurs, the likelihood of sudden collapse and death is always present.

The Heart Enlargement is often noticeable in some degree in severe cases during examination in the second week, and is likely to be greater in fatal cases. Evidence of right-sided failure was found in one case in which oedema, ascites, enlargement and tenderness of the liver, venous congestion, and oliguria occurred. Extra systoles were encountered in two cases only.

Pericarditis In six cases at postmortem examination clear fluid was found in the pericardial sac, in one case associated with pericardial adhesions. These changes were noted by HICKS (1945) in seven out of 24 postmortem examinations in which over 100 c.c. of fluid was found. Pericarditis was not diagnosed during life in this series.

Myocarditis Apart from right-sided dilatation of the heart, macroscopic changes are seldom found in mite typhus, though microscopic changes are usual (HICKS, 1945, BERRY *et al*, 1945). Electrocardiographic investigations have revealed no evidence of myocardial changes either in the course of the disease or in convalescence. WILLIAMS *et al* (1944), in their investigation of 600 cases in New Guinea, report no changes apart from one case with prolonged P-R interval and another with pulsus alternans. The findings in nine cases, together with 21 convalescents investigated by BERRY *et al*, were also negative.

The Local Sore or Eschar

In 99 cases out of 450, in which accurate records were kept, local lesions were found. The distribution was as follows: axilla, 34 per cent, abdomen, 26 per cent, back, 20 per cent, limbs, 13 per cent, neck, 7 per cent. In 26 of these cases rashes were also observed. It should be noted that a thorough search for eschars is necessary before exclusion, the whole body must be examined with particular reference to the genital organs, perineum, buttocks and scrotum. It is probable that more local lesions would have been found in the present series if more thorough examination had been undertaken at the expense of the comfort of these very ill people. In the circumstances of the outbreak in 1944 diagnosis was easy, and therefore search for minutiae in physical examination was sometimes sacrificed for the patients' benefit.

The existence of multiple eschars was noticed on two cases in this series. Multiple bites are described by KITASHIMA and MYIAJIMA (1918) and MENON and IBBOTSEN (1945).

The local ulcer of tsutsugamushi disease has been ably described by numerous writers. The first stage is a papule at the site of the bite. There is no local irritation

or part of any severity but probably by friction a shallow ulcer is formed about 5 mm. in diameter oval in shape, surrounded by a red areola (Stage 2). The patient is seldom seen in this Stage 2. By Stage 3 scab formation has taken place over the ulcer and the inflammatory signs are subsiding. Most of the cases with local lesions are first seen at this stage. If seen in the first 4 days of fever Stage 2 may be seen. In the present series only a very small proportion seen at this stage of the fever had icera.

The inflammatory changes have now disappeared and the site of the red areola is marked by scaling. The central scab is shed in 2 or 3 weeks.

The patient never seeks advice on account of the sore; the bite is not painful or irritating and it is not remembered with any clarity. For this reason the early lesions are seldom seen.

Among those working in infested country frequent bites are sometimes seen. Some of these cause irritative lesions only (scrub itch) but no primary lesion (GUTHRIE, 1938).

The Japanese originally considered the sore as an essential clinical feature of tsutsugamushi fever. It was for this reason that FLAHERTY originally recognized three diseases in the Malay States: Tsutsugamushi fever (with sore), rural typhus and urban typhus (without sore). The cases without sores were regarded as distinct from tsutsugamushi fever until LAWRENCE and SAVOOR (1936) demonstrated the essential identity of the two diseases. Their explanation of the frequent absence of the skin lesions was as follows.

() Healing may take place before the patient comes under medical observation. This explanation is especially feasible in view of the variable incubation period of the disease; the variable local response to insect bites in different individuals and the variable sensitivity of the skin. They suggest that the dark-skinned Tamil wearing few clothes has less sensitive skin than the European who is habitually fully dressed.

(b) The healing lesion is difficult to detect in dark-skinned Asiatics, although the difference in incidence of the primary lesion between the two groups is not entirely explained in this way.

() Experimentally intradermal injection of virus causes a local papule followed by a sore; subcutaneous injection caused no local lesion (NAGAO *et al.*, 1923; LAWRENCE and SAVOOR, 1936). The theory is that the mite in nature may inject rickettsiae intradermally or subcutaneously depending possibly on the length of the proboscis. In the former case the local lesion may arise; in the latter there would be no skin lesion.

On these lines it is not difficult to explain the wide variation in incidence of local lesions in reports by different writers. (HARRIS 1915 34 per cent. LAWRENCE and SAVOOR, 1940 5 per cent. ADAMS and EVANS, 1948, 78 per cent. SINCH 1945 40 per cent.)

The Rash of Mite Typhus

The rash is seen most commonly and in most striking form in Europeans at its earliest on the fourth or fifth day of the illness but may appear at a later stage. It commences on the chest or abdomen, spreading thence to the neck, face and limbs. It is most pronounced on the trunk and sometimes the face is unaffected. At first it consists of macules often small in size, increasing in size and number till they are widespread, giving a mottled appearance. If they arise first on the abdomen they may easily resemble the rose spots of typhoid, but their subsequent spread to the face and neck may cause suspicions of varicella or variola—a vesicular stage never appears in typhus. When widespread, the rash may take a variety of forms, consisting of large and small macules and papules or a diffuse erythema with super added macules and

papules The abdomen, chest, back, forehead and cheeks, neck, and limbs are affected, the palms and soles are unaffected. In Indians with moderately dark skins the rash resembles that of Europeans. In black-skinned Indians and Africans, the rash is less commonly visible, is probably of less intensity, and must be looked for carefully. Often it takes the form of a diffuse erythema only. The skin appears to be puffy and "swollen," the hair follicles are swollen and the glossy appearance of the healthy skin is absent. The skin feels hot and dry, sweating being absent or minimal.

The rash reaches its maximum intensity within a few days and gradually fades, leaving some brownish discoloration. The fading is succeeded by peeling, this is an important sign in dark-skinned subjects, in whom it is easily detected. In several askaris peeling of the skin and back has been the only evidence that the rash had been present, the rash previously not being noticeable. It should be carefully searched for during the second and third weeks in all cases.

Facial Appearance—The characteristic appearance of the face as "bloated or puffy" has been noted by several writers notably MEGAW (1945). Flushing is noticeably present in European cases, and oedema of the eyelids is usual. Cyanosis is an indication of severe cardiovascular involvement and is commonly present in severe cases. Circumcorneal injection and oedema of the conjunctivae are also striking features.

Blood

In 25 consecutive cases in which leucocytes were counted, the average total leucocyte count was 6,450 white blood cells per c mm. The maximum reading was 10,000 white cells in a case with broncho-pneumonia. On the whole, the leucocytes tend to be reduced in uncomplicated typhus and increased in number in the presence of septic complications. Differential counts show no unusual features. These findings are in agreement with most authors.

Malarial parasites in thick films were found in 63 cases, M T 45 (10 per cent), B T 18 (4 per cent).

Spleen

The spleen was palpable in 60 per cent of the cases but the size was never very great except in certain African and Indian subjects in which previous malarial attacks had caused the spleen to be enlarged and firm. In these cases further enlargement occurred during the course of the fever.

Lymphadenopathy

Is so constant a feature as to be almost characteristic and was present in 90 per cent. of all the cases by the end of the first week. The glands are most noticeably palpable in the posterior triangles of the neck, but some

enlargement of glands in the axillae and groin is usual and the epitrochlears can usually be easily felt. In a few cases all the cervical glands were grossly enlarged, including the sub-mental and sub-maxillary groups, giving a 'bull necked' appearance. In these cases considerable pain and discomfort in the neck and pain on swallowing is complained of. Enlargement of posterior cervical glands may be a cause of the occipital pain which is occasionally associated with neck rigidity.

Before generalized enlargement occurs, the glands draining the region of the primary sore are often enlarged. They are painful and tender and abscess formation may occur.

Duration of Fever

The average duration of fever was 17.2 days in 400 cases of uncomplicated typhus. The duration of the fever is prolonged in the presence of complications such as bronchio-pneumonitis, pleural effusion, abscesses, thromboses and bed sores.

Subsidence of fever was usually by lysis. In six cases the fever terminated by crisis. In these cases there is a risk of sudden collapse at this period and death may occur (KLEIN 1945). In the present series there were four such cases, in two of which death occurred.

The Weil Felix Reaction

In the first 100 cases in which the serum was tested against *Proteus* OXK, there were 63 cases in which agglutination occurred at titre of 1:125 or higher. The 37 negative cases included those in which diagnosis was not at all in doubt on clinical grounds. In some of them an eschar was seen, in others typical rash, and in five cases death occurred.

From this it may be inferred that single Weil Felix reaction, unless positive at titre of 1:125 or higher is of little diagnostic value. This test must be repeated at weekly intervals for several weeks to detect rising titre which is of special significance (MIRMAN 1945). In the circumstances in which this series of cases was treated in forward hospital, eradication of cases was imperative in all cases as soon as the patients' condition permitted; that is to say as soon as the febrile period had ended and the general condition showed signs of improvement. It was accordingly impossible to follow up patients and to record serial Weil Felix reactions except in several cases. The standard titre of 1:125 as suggested by LEVITZWARTZ for single test, in cases where clinical features were strongly suggestive, was accepted as positive. For these reasons the Weil Felix reaction in the Lumphal area was of very limited value in the circumstances of the campaign because it was found, firstly that the reaction was commonly negative before the 12th day of disease; secondly that the titre of agglutination sometimes increased late in the course of the case and sometimes in convalescence. For example one case under the care of another physician was shown to the writer where serial weekly Weil Felix reactions throughout the illness were negative but 1:1,500 titre agglutination occurred in the fifth week from the onset. In this series the maximum agglutination titre recorded was 1:12,500 OXK on the 19th day having been 1:140 on the ninth day. This case illustrates the great change in titre which may occur in a comparatively short period.

Study of the Weil Felix results reveals that the titre of agglutination bears no relation to the severity of the infection. Severely ill patients and fatal cases are found in which the reaction is entirely negative.

TABLE III
LIST OF CLINICAL FEATURES AND VALUES AUTHORITY

Clinical Feature	Burma		Africa		Asia		Europe	
	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent
Headache	100	100	100	100	100	100	100	100
Stiff neck	100	100	100	100	100	100	100	100
Generalized rigidity	100	100	100	100	100	100	100	100
Chills	100	100	100	100	100	100	100	100
Continuous fever	100	100	100	100	100	100	100	100
Rash	100	100	100	100	100	100	100	100
Diagnosis	100	100	100	100	100	100	100	100
Duration	100	100	100	100	100	100	100	100
Death	100	100	100	100	100	100	100	100
Chief	100	100	100	100	100	100	100	100

* Headache commonest feature

CLINICAL FEATURES IN 1,200 CASES IN ASIA AND BURMA

Table III is designed to show the main features of the disease described by various authors. It will be noted that the features described in this series of cases correspond closely to those of *Leishmaniasis* cases which were observed in the same area on the Asian-Burma border in the previous year.

The series, comprising nearly 1,200 cases, may fairly be said to represent the disease as it occurs in Burma in males between the ages of 20 and 40 years.

COMPLICATIONS The following complications were encountered —

1. **Respiratory System**
 - Pharyngitis, laryngitis, bronchitis
 - Pulmonary consolidation or pleurisy
 - Lung abscess

75 per cent
22 cases
2 cases

2. *Cardiovascular System.*

Cardiovascular failure, acute	4 cases.
subacute			15 cases.
extrasystoles	cases.
Pericarditis (postmortem evidence)	6 cases.
Venous thromboses (one axillary one femoral)	2 cases.

3. *Nervous System.*

Cerebral toxæmia manifested by coma, convulsions			
incontinence mictæ, meningismus	14 cases.
Deafness	93 cases.

4. *Hæmorrhagic.*

Epistaxis	1 case
Purpura			2 cases.

5. *General.*

Concurrent malaria M.T.	43 cases.
" B.T.	18 cases
Parotitis (one parotid abscess)			5 cases.
Bed sores	4 cases.
Abscesses	2 cases.
Hypertyreism (terminal)	2 cases.

DISCUSSION

The disease is accompanied by marked weakness and wasting which is soon remedied by prolonged rest and satisfactory dieting. In no case in this series was retention in hospital for longer than 2 months possible, and the majority were evacuated as early as safety permitted. For this reason a follow-up of cases was not, in the circumstances prevailing, possible. Patients during convalescence quickly regained weight and strength, though tachycardia and dyspnoea on exertion were noticeable for a period. LAYTON (1945), in his survey of 118 convalescents, found 49 in whom the effort syndrome was present. In no case were abnormal physical signs or electrocardiographic changes found. Similar absence of abnormal signs of cardiac involvement was noted by BERRY *et al.* (1945) in nine cases during the course of the fever and in 21 convalescents. These authors stress the point that suggestion of heart disease may make harmful impressions on the minds of convalescents. One reason for this is that, among European troops in Burma, for example, typhus acquired a reputation for its high mortality supposedly due to heart failure. Sufferers aware of the nature of the condition were often apprehensive both during the course of the disease and in convalescence.

MORTALITY

Table IV illustrates the variation in mortality rates experienced by various authors. Study of the reports in different outbreaks in the campaign in the Far East indicates that there was great variation in the circumstances in which the

TABLE IV
FATALITY RATE DATA RECORDED BY VARIOUS AUTHORS

Authors	Area	Number of cases	Mortality percentage	Period
KITAHARA & MIYAJIMA (1918)	Japan, S. Korea	1,200	8	1914-1915
CORREIA & WHITECOX (1944)	India, S. India	85	14	1943-44
LAWTHWAITE (1936)	India, S. India	798	14	1936-37
ANDERSON (1941)	India, S. India	78	1	1941-42
SCHULTZ (1941)	India, S. India	107	17	Sept. 1941
ARMSTRONG & LYNCH (1946)	India, S. India	8	10	Nov. 1943-Sept. 1944
		(Ceylon)		
TAYLOR (1941)	India, S. India	700	77	Jan. 1942-Jan. 1943
MURPHY & LYNCH (1944)	India, S. India	110	82	Nov. 1944-Jan. 1945
WHITECOX	India, S. India	427	87	Jan. 1944-45
*WHITECOX (1944)	India, S. India	600	7	1944
	Ceylon, epidemic	7	17	Jan. 1944 figures
LAMB (1941)	India, S. India	1,54	8	July 1942-Sept. 1943

* Official War Office figure.

† Proceedings of a conference at H.Q. Ceylon Army Command, February 1944.

disease was treated and in the condition of the troops at the time of exposure. For example, WHITEHEAD *et al.* (1944) in their study of 626 cases in New Guinea, considered that the fatality rate was profoundly influenced by lowered resistance from hardship, malaria and dysentery.

The cases described in this paper were among troops engaged in jungle warfare under severe conditions of hardship, sometimes inadequate rations, limited sleep, exposure to rain and nervous tension. In addition, their journeys to hospital though perhaps not more than 30 to 50 miles, often occupied 2 days' travelling on account of the poor conditions of the roads in the monsoon. Nevertheless, it is clear that even in static conditions, the fatality rate varies widely. For example, the figures recorded by LAWTHWAITE (1936) and others differ widely from those of the Ceylon epidemic (1944). LAMB (1945) states: "The mortality rate is found to vary considerably in different areas from 0 to 30 per cent, and this appears to be due to variation in virulence in the strain of rickettsia rather than to variable dosage." The same view was expressed by KITAHARA and MIYAJIMA (1918), who noted great variation in fatality rate according to district.

For restlessness and mental excitement morphine may be given with benefit provided the pulmonary condition is good, but paraldehyde is the safest and most useful drug to employ.

When the symptoms and signs point to involvement of the lungs, symptomatic treatment on the same lines as for pneumonia is called for except that sulphonamide drugs should be avoided. The latter should only be employed

TABLE V
THE EFFECT OF SULPHATHIAZOLE ON MITE TYPHUS CASES WITH PULMONARY SIGNS.

Case	Day of disease drug given.	Temperature normal on.	Grammes given.	Result.	Outcome of case
1	10-14th	14th	21	Good effect of drug doubtful	Recovery
2	10-14th	17th	21		"
3	13-18th	18th	20		"
4	14-18th	20th	21		"
5	13-18th	20th	21		"
6	6-8th	16th	16		Recovery
7	11-10th	18th	20		"
8	11-10th	20th	21		"
9	2-6th	13th	26		"
10	7-10th	20th	21		"
11	8-12th	22nd	28	Drug without effect	"
12	2-9th	12th	21		"
13	6-9th	19th	1		"
14	8-11th	—	21		Death 14th day in hyperpyrexia. Pulmonary congestion.
15	7-11th	—	26		Death 12th day Toxaemia and pulmonary congestion.
16	6-9th	—	21		Death 10th day Broncho-pneumonia
17	8-12th	—	26		22nd
18	8-10th	—	11		23rd Toxaemia and pulmonary congestion.
19	10-12th	—	21		Death on 14th day Purulent bronchitis and broncho-pneumonia, and L. dry pleurisy

Dosage First day, grammes 8 subsequent days, grammes 2 daily

when there is evidence from examination of the sputum of secondary infection of the lungs by pyogenic organisms. These drugs, apart from the risk of causing renal complications, increase the degree of toxæmia. Table V shows the effect of sulphathiazole in cases of typhus with pulmonary signs. In four cases the temperature fell by 1/2° after its use, but in no case was it certain that the result was not due to the natural course of the fever rather than to the effect of the

drug In 14 cases the drug had no beneficial effect and in two of these cases the drug was withdrawn owing to increase in the signs of toxæmia AGRESS and EVANS (1946) also reported that sulphathiazole administration added to the discomfort of the patients

Penicillin—KLEIN (1945) and BLAKE *et al* (1945) found that penicillin had no effect on the course of the illness, as would be expected on theoretical grounds Similar findings were apparent in the few cases in which it was possible to try its use in Burma

PROPHYLAXIS WITH VACCINE

Field and laboratory experience with vaccine in the control of epidemic typhus showed that immunization in the Mediterranean area effected a lessening in the severity and a reduction in mortality, while there was evidence that, although anti-lice measures were employed concurrently, the incidence of the disease was lowered

Efforts to employ vaccine in the control of mite typhus in S E A C were too late to be brought into action before the end of hostilities Work was started at the National Institute for Medical Research, London, culminating in the production of cotton-rat lung vaccine capable of protecting laboratory animals (FULTON and JOYNES, 1945, BUCKLAND *et al*, 1945) It arrived in Burma in small amounts only, in July, 1945, at a time when the disease, though present, was declining in incidence, and was not nearly so prevalent as in 1944 at the same time of year Moreover, the capture of Rangoon in May, 1945, was soon followed by sudden cessation of hostilities over large areas of Burma, units were on the move, many being destined to proceed to Malaya, resulting in delay and difficulty in carrying out controlled tests

CONCLUSIONS

The clinical features of the disease in the present study indicate that the malady popularly known as scrub typhus, is clinically identical with tsutsugamushi fever of Japan and the Far East It is considered that mite typhus is the most suitable designation

The disease is known to have been endemic in India, Assam and Burma since 1935, but cases were so few that little interest was attached to the disease In the early part of the recent war in the Far East the incidence increased greatly over scattered areas, and captured documents show that the Japanese forces were affected, probably fairly severely The incidence in our troops in Burma rose in 1943 and 1944, reaching a climax in the summer and autumn of 1944, after the siege of Imphal This outbreak is believed to be the largest recorded in history in one area The retreat and defeat of the Japanese Army in 1944 in Burma was brought about by the success of our forces consisting of Indian, African and British troops, who were exposed to infected "scrub"

country on an extremely wide scale. The occurrence of the disease on such a great scale was one of the outstanding medical features of the Far Eastern war and one of the most important problems concerning the military medical organization.

The pathology is almost identically the same as that of louse borne (epidemic) typhus, although the epidemiology is entirely different. It is, therefore, to be expected that the clinical features of the two conditions are closely similar. Study of cases shows this to be the case. This study reveals that there is no essential difference between the clinical features in British, Indian and African subjects, although the cutaneous manifestations vary according to the degree of pigmentation of the skin in each individual patient.

There is also slight variation in the mortality rate probably due to the variation in the degree of natural immunity.

The Weil Felix reaction was employed in diagnosis, but in the difficult circumstances of the campaign it was found that clinical diagnosis was relatively easy practical and safe.

At present there is no specific treatment for the disease, although hope is entertained that the use of drugs of the para-amino-benzoic acid group in the early stages will meet with success. The same hope is held with regard to the employment of vaccine in prophylaxis.

There is no evidence that either penicillin or sulphonamides have any effect on the course of the disease though benefit occurs in pulmonary complications due to secondary infection by pyogenic organisms. Sulphonamides should, in general be avoided because they increase the degree of toxæmia and may cause renal and other complications.

SUMMARY

1. The recognition of mite typhus fever in India, Assam and Burma in recent years is discussed. Its low endemicity before the recent war against Japan was such that until after 1930 it was unrecognized. The great importance played by the disease in the Burma campaign is described.

2. The clinical findings in 493 cases seen in Assam and Burma from 1944 to 1946 are discussed and compared with those of other authors. The majority of cases were in East African troops. The disease is clinically identical with tsutsugamushi fever as seen throughout the Far East and in Japan. There is no essential difference in the features of the disease as seen in East African Indian and British subjects.

3. The Weil Felix reaction is often of no value in diagnosis, as agglutination at a diagnostic titre is rarely found before the 12th day and frequently may not be obtained until the patient is convalescent. Clinical diagnosis in the second week or earlier is usually easy.

4 The clinical findings and macroscopic findings at postmortem show that the disease is clinically closely similar to louse-borne epidemic typhus, though epidemiologically they are so different

5 There was no specific treatment for the disease though hope is now entertained that the use of drugs of the para-amino-benzoic acid group will be highly beneficial

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he was treated for malaria and dysentery. He failed to respond, and died at 4 p.m. on 9th March. An autopsy was performed at 9 a.m. next day.

POSTMORTEM EXAMINATION

The body was wasted, the conjunctivae were yellow.

The surface of the *brachia* (Fig. 1) showed a slightly depressed area, 5 cm. \times 3 cm., in the lateral aspect of the right parieto-occipital region. The pia-arachnoid over it was haemorrhagic, and there were also outlying petechiae. For some distance around, the subarachnoid fluid was yellowish. There was no obvious meningitis. A coronal section at this site showed the haemorrhagic lesion to penetrate the brain to a depth of 2.5 cm. The cerebral tissue immediately around the haemorrhagic area was brown and slightly softened, that further out yellowish. The brain in general was oedematous. It weighed 1475 grammes.

The *heart* (185 grammes) showed toxic spalling, and there was an excess of yellowish pericardial fluid. The *lungs* showed consolidation in the lower lobes and parts of the upper lobes: the cut surface was deep red and felt nodular. There were numerous petechiae in the pleura, and in the substance of the lungs. The left pleural cavity contained 80 ml. of yellowish-brown fluid, the right 30 ml. The tracheo-bronchial lymph nodes were acutely inflamed.

The *stomach* showed numerous shallow ulcers (Fig. 2). Many were rounded, about 8 mm. in diameter: others were irregular: many were confluent. The floor of the ulcers was grey: the sharply sloping sides red or black, the edges well defined, not raised. There was no inflammatory zone around, apparently normal gastric mucosa extending to the very edge of the ulcers.

In the *small intestine* at about the midpoint, was an ulcer 15 mm. \times 10 mm. with raised, yellow edges and sunken rough, congested floor. In the jejunum were several white nodular thickenings in the mucosa about 3 to 5 mm. across. Peyer's patches were not inflamed.

The *large intestine* showed seven rounded ulcers, the largest measuring 18 mm. \times 12 mm., the smallest 10 mm. \times 10 mm. Their general appearance was similar to that in the small intestine except that the heaped-up edges were deep red in colour (Fig. 3). The overlying serosa was not affected. The seven ulcers were distributed singly in the caecum and ascending and transverse colon. The intervening mucosa showed no signs of inflammation. The distal part of the large intestine including the sigmoid colon and rectum, was quite different in appearance. The mucosa was diffusely inflamed and dark red, and there were many irregular superficial ulcers covered with yellow slough. The serosa was injected. This severely inflamed area, interpreted as indicating bacillary dysentery extended to about 9 cm. from the anus.

Lymph nodes in the mesentery and in both inguinal regions were enlarged and inflamed.

The *peritoneal cavity* contained 350 ml. of yellowish-brown fluid.

The *liver* (1,330 grammes) was soft, patternless, brown. It contained no visible abscesses. The *gall bladder* was filled with white bile. The cystic duct was not obstructed. The *spleen* (270 grammes) was rather enlarged, brown on section and slightly soft: its Malpighian bodies were only slightly noticeable: no organisms were detected in it by smears or cultures.

The *kidneys* (each 130 grammes) were yellow and toxic, and showed areas of purulent inflammation mostly in the form of radial lines in the cortex. The renal pelves were not inflamed. The *suprarenals*, *testes* and *pancreas* appeared normal. There were ecchymoses in the mucosa of the *urinary bladder*.

HISTOLOGICAL NOTES

Specimens from the following tissues were fixed in 10 per cent. formalin in isotonic saline, embedded in paraffin, stained with haematoxylin or iron haematoxylin, and usually counterstained with eosin.

Bram A sulcus is present in the centre of the section. The pia mater within it is densely infiltrated with cells, the great majority of which are neutrophil leucocytes. There are areas of haemorrhage, and areas of necrosis. In the latter are many fragments of necrosed leucocytes.

Amoebae are scattered copiously throughout the pia and are massed in places especially around arterioles. On either side of the sulcus large numbers are invading the cortex (Figs 4 and 5). They are often utilizing perivascular pathways. Some amoebae lying in the adventitia of arterioles are very minute—as small as 3μ in diameter. Haemorrhage and necrosis can readily be understood, for in places arteriolar walls are disintegrating before the amoebic attack, although the muscle of the media is comparatively resistant (Fig. 6).

For the most part the amoebae in the brain substance are exerting no cellular resistance, they are simply dissolving the cerebral tissue as they advance. Here and there a few phagocytes are making a brave attempt to stem the flood of invasion (Fig. 14). In places where haemorrhage has occurred, the amoebae are greedily ingesting the red cells (Fig. 15), as many as five may be seen in one amoeba (not illustrated). Other amoebae contain granules staining more or less deeply with haematoxylin which probably are fragments of tissue. In many places there are many small granules scattered among the amoebae which are no doubt remains of partly lysed tissue or of disintegrating amoebae.

Small intestine (ulcer) Ulceration has destroyed the mucosa and exposed the submucosa. The immediate floor of the ulcer consists of necrotic material, and beneath this is a layer of inflammatory tissue reaching to, and in places penetrating, the *tunica muscularis*. The framework of this tissue is provided by the sturdier tissue elements which have resisted inflammatory destruction, this is filled out with inflammatory cells, mostly neutrophil leucocytes, many of them fragmenting. Fibroblastic proliferation is present in only a few places. Throughout the inflammatory tissue are multitudes of amoebae (Fig. 7). A broad band of them, up to 30 deep, underlies most of the ulcer just beneath its floor, elsewhere they lie scattered or in small groups, occasional individuals have penetrated the muscularis or ventured far out in the submucosa beneath the normal mucosa. Amoebae, together with leucocytes, are within the lumina of some venules (Fig. 7) and lymphatics. The thicker walls of arterioles have enabled them better to withstand invasion, though some are necrosed.

Small intestine (nodular thickening) The section shows this also to be a site of amoebic invasion. Shallow ulceration has destroyed the mucosa, and in the submucosa beneath is an inflammatory mass, through and beyond which numerous amoebae are scattered. The infiltrating cells are nearly all neutrophils. There is little necrosis of tissue, and no fibroblastic reaction. The appearance is similar to that of the previous section, except that the process is at an earlier stage. A lymphatic and a venule containing amoebae and inflammatory cells are shown in Fig. 9 and Fig. 17 respectively, and groups of amoebae in Figs. 18 and 19.

Stomach The section is made through an ulcer, which has destroyed the full thickness of the mucosa and penetrated the submucosa. The histological picture is in general similar to that of the small intestine. Amoebae are scattered throughout the base of the ulcer and become numerous in places at and beyond the edge of the ulcer, where masses of them lie among the tubules (Figs. 10 and 11). Many lymphatics contain amoebae, together with pus cells, and walls of venules and arterioles are being destroyed.

Caecum The histological picture of the caecal ulcer is again similar. Amoebae are numerous and lie scattered or grouped. The *tunica muscularis* is being actively invaded.

Sigmoid Colon The section includes an area of ulceration which has destroyed the mucosa. The ulcer is floored with necrotic tissue covering a zone of neutrophil leucocytes. The adjacent submucosa is congested and haemorrhagic, and lightly infiltrated with cells which include neutrophils, lymphocytes and histiocytes, there is some proliferation of fibroblasts. The amount of inflammatory reaction is not great suggesting a feeble defence against the invading organisms. No amoebae could be found. The histological examination is in accordance with the macroscopic diagnosis of bacillary dysentery.

Mesenteric lymph node The node is intensely inflamed and there are extensive areas of partial or complete necrosis. Of the normal lymphoid tissue there remain a few

small irregular foiboles and here and there diffuse scattering of lymphocytes. Neutrophil leucocytes abound, many of them fragmented. Histocytes are filled with nuclear debris.

Throughout the node are amoebae lying singly or in small groups. In places they are massed in close formation (Figs. 1 and 15). The amoebae constituting the masses tend to be smaller in size than the scattered ones—probably from rapid multiplication—and their cytoplasm more lightly stained.

The inflammation has spread beyond the node. The surrounding mesenteric tissue is oedematous and thickly infiltrated with inflammatory cells—mostly neutrophils—among which lie numerous amoebae. Large lymphatic vessels near the node capsule are packed with pus cells and contain occasional amoebae.

Gastric lymph node. The node and surrounding tissues show intense, acute inflammation with necrosis and neutrophil infiltration as in the mesenteric node. There are areas of haemorrhage. Amoebae are present singly and in small groups. In one area they are being actively ingested by histocytes.

Right lung. The section includes an inflammatory area 3 mm. across, in which the alveoli are largely filled with necrotic cellular debris. In places there is vigorous neutrophil reaction. Alveolar walls are thickened and are becoming obscured from involvement in the inflammatory process. Amoebae are present, being more numerous at the margin of the area (Fig. 8). They are also noticeable in the adventitia of several arterioles, from whence they are invading and breaking up the media; the lumina still contain blood. Elsewhere in the section the pulmonary tissue shows patchy inflammation, various groups of alveoli containing serum, red blood cells, mononuclear cells, neutrophils, or combination of some of these. Scattered amoebae are to be found in the inflammatory foci.

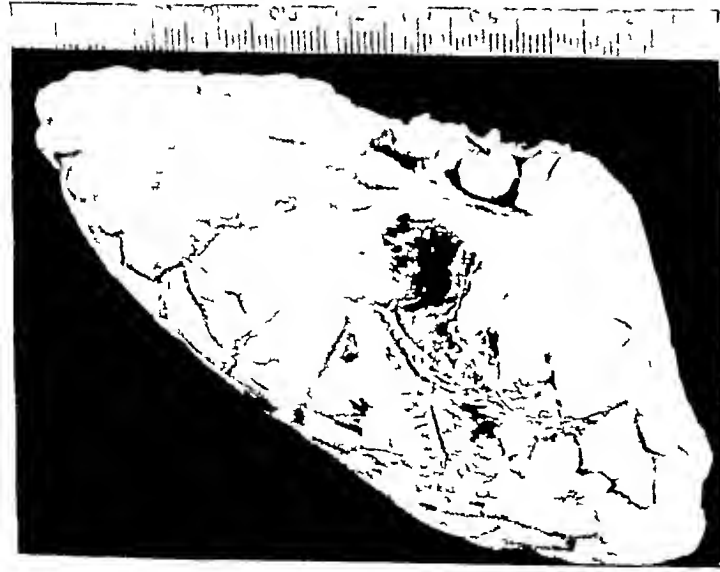
Left lung. The section shows patchy inflammation similar to that described in the right lung. Amoebae are present, but scanty.

Liver. There is some increase in periportal fibrous tissue and occasionally periportal lymphocytosis. Some lobules are rather irregular in shape and size. The hepatic cells are separated from one another (perhaps postmortem change). Some cells are quite large and contain enlarged or multiple nuclei. In other cells the nuclei are degenerating. The cytoplasm is granular. Some cells contain small brown masses, presumably of bile. There is much dark brown, coarse pigment in the Kupffer cells. There are no macro-abscesses in the section, nor are amoebae to be seen.

Spleen. The Malpighian bodies are small with no germinal centres. The pulp is congested and shows great increase of nucleated cells. Many cells contain coarse granules of brown pigment. There are small haemorrhages, but no necroses or abscesses. No amoebae could be found.

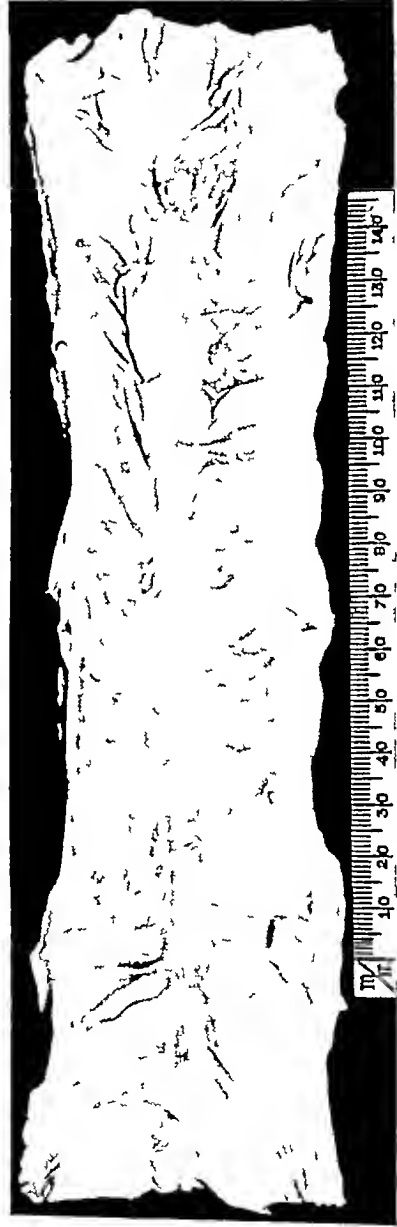
LEGENDS TO ILLUSTRATIONS.

- FIG. 1. Right parieto-occipital region of brain. Haemorrhagic amoebic lesion in substance of brain and in pia-arachnoid.
- FIG. 2. Portion of posterior wall of stomach including pylorus, showing multiple amoebic ulcers.
- FIG. 3. Portion of colon, showing three amoebic ulcers.
(In Figs. 2 and 3 the photographs have been touched to define the ulcers more clearly the colours of the specimens having faded.)
- FIG. 4. Masses invasion of brain by amoebae. Nearly all the visible cells are amoebae. They appear to be dissolving the cerebral tissue without, at this site, provoking any inflammatory reaction. Many amoebae contain red cells, which are demonstrated more clearly in the higher magnification shown in Fig. 12. $\times 350$.
- FIG. 5. Another example of mass invasion of brain by amoebae. T. capillaries are withstanding dissection. $\times 350$.
- FIG. 6. Arteriole of brain disintegrating before amoebic attack. Most of the lumen is occupied by thrombus. In the left lower part some free red cells persist. The wall is being eroded by amoebae and replaced by inflammatory tissue in which fragmented leucocytes abound. $\times 40$.
- FIG. 7. Inflammatory tissue beneath base of ulcer of small intestine. The illustration includes vessels (V) filled with inflammatory exudate in which are several amoebae and an arteriole (A) the walls of which are necrotic. Numerous amoebae are to be seen in the tissue spaces, especially in left upper quadrant. $\times 250$.

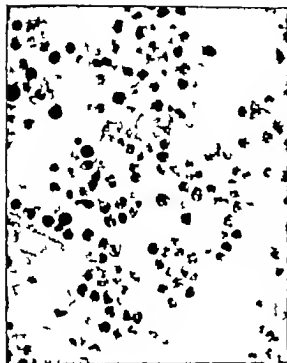


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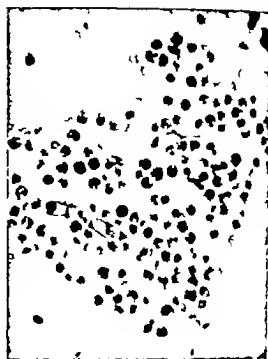
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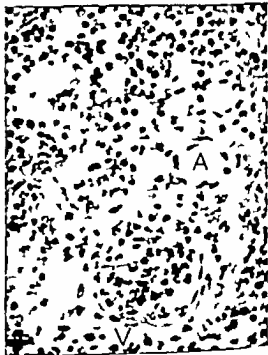
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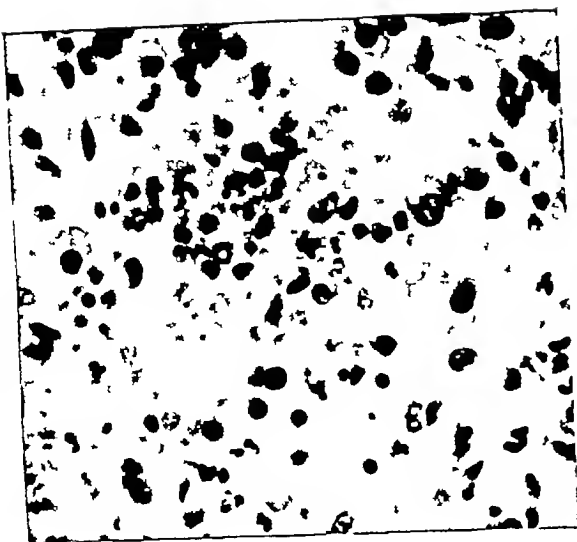


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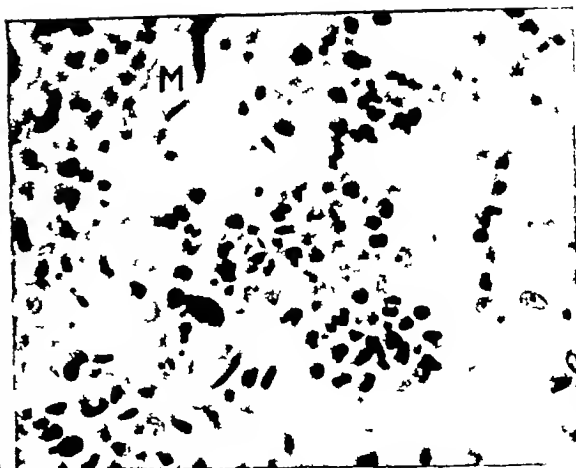


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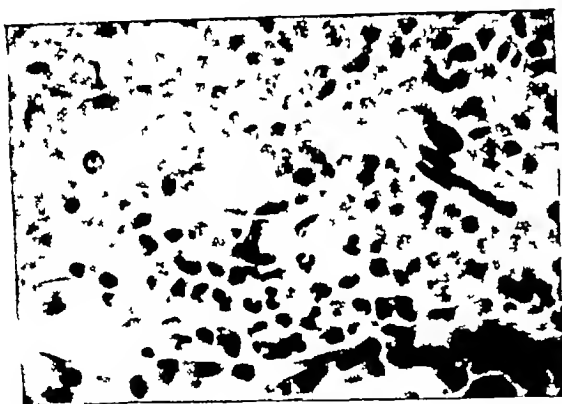




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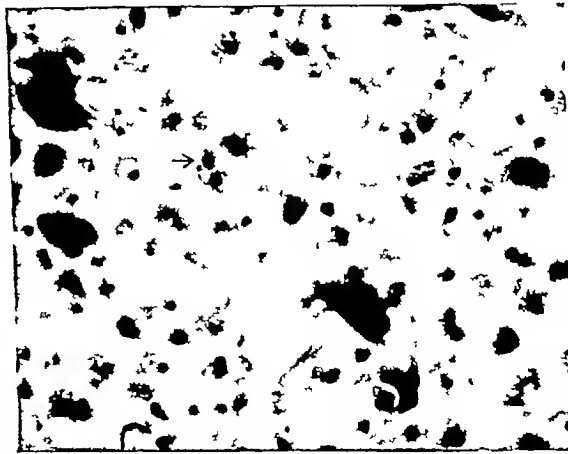
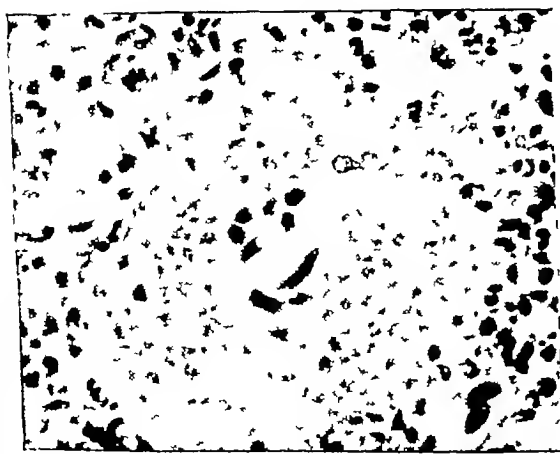
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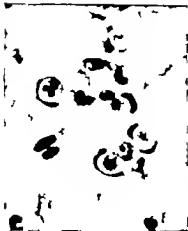


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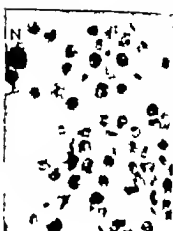




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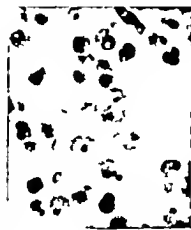
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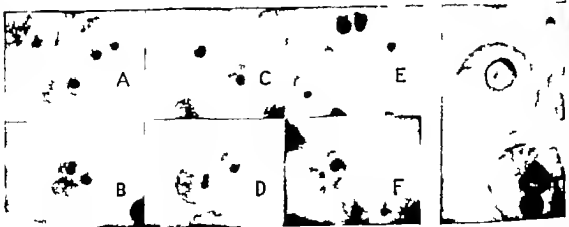
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Left kidney Sections show numerous small abscesses, often confluent. Most are in the cortex, but many are in the medulla. They consist of neutrophils, actively phagocytic histiocytes and necrotic cellular debris. Lymphocytes abound at the margins. In one abscess haemorrhage has occurred. Nearby tubules contain leucocytic casts. Glomeruli appear normal, and vascular thromboses are not evident. No amoebae could be found in spite of a prolonged search. In the renal tissue away from the abscesses, the notable features are toxic spoiling of the proximal convoluted tubules and the presence of yellow-brown homogeneous material in some of the distal convoluted tubules.

THE MORPHOLOGY AND IDENTITY OF THE CAUSATIVE AMOEBA

The presence of amoebae, often in enormous numbers, is the most significant feature in the histology of this case. These amoebae show the same morphology in all the tissues wherein they are found. In size, as measured in the stained sections, they vary considerably—the extremes being 3μ and $12 \times 9\mu$, and the average about 6 to 8μ . The average size is about that of the neutrophil leucocytes present in the sections (see, for instance, Figs 7 and 9).

The cytoplasm is granular or foamy. It occasionally includes small vacuoles. Ingested red blood cells are frequently to be seen, especially in the brain lesion.

LEGENDS TO ILLUSTRATIONS

- FIG 8 A pulmonary alveolus containing amoebae and inflammatory exudate. Three amoebae, one of which has a double nucleus, are shown at higher magnification in Fig 20A. $\times 510$
- FIG 9 A submucosal lymphatic in the small intestine containing amoebae and inflammatory exudate. *M. muscularis mucosae*. A higher magnification is shown in Fig 20E. $\times 480$
- FIG 10 Massing of amoebae in mucosa of stomach. A tubule lies along the lower margin. $\times 510$
- FIG 11 Massing of amoebae in mucosa of stomach. A tubule is seen on the left and a branching arteriole on the right. $\times 760$
- FIG 12 Periaarteriolar massing of small amoebae in a mesenteric lymph node. Many amoebae in this field measure only 3 or 4μ in diameter. $\times 500$
- FIG 13 A group of small amoebae in a mesenteric lymph node. The small size is ascribed to rapid multiplication. Several amoebae contain two nuclei and one, indicated by an arrow, a red cell. $\times 1,040$
- FIG 14 Attempted phagocytosis of amoebae in brain. Five amoebae have each become encircled by phagocytes, probably cerebral histiocytes. $\times 820$
- FIG 15 Active ingestion of red blood cells by amoebae in brain. The arrows point to ingested red cells. Free red cells are shown at R. $\times 820$
- FIG 16 A group of amoebae in the brain. A nerve cell is shown at N. $\times 820$
- FIG 17 A submucous venule containing amoebae in the small intestine near a nodule. The readiness of amoebae to enter venules and lymphatics explains their widespread dissemination. $\times 480$
- FIGS. 18 and 19 Submucosal groups of amoebae of varying sizes in a nodule in the small intestine. The largest amoeba measures about $11 \times 9\mu$. $\times 1,040$
- FIG 20 A composite picture to illustrate various nuclear appearances. The characteristic large karyosome is shown, separated by a clear zone from a delicate nuclear membrane. On the left are two examples of double nuclei, A from lung, B from small intestine. The kidney-shaped karyosome in C (from small intestine) and the hour-glass shape in D (from brain) are probably dividing forms. The two amoebae on the right are probably somewhat degenerate, in E (from small intestine) the clear zone has become obscured and in F (from stomach) the nuclear ring is unevenly stained. $\times 1,700$, except D which is $\times 1,600$.
(The sections from which photomicrographs were taken, except Fig 21, were fixed in formal-saline, stained with Ehrlich's haematoxylin or iron haematoxylin, and usually counterstained with eosin.)
- FIG 21 *Entamoeba histolytica* from a fatal case of intestinal amoebiasis, for comparison. Zenker fixation, H & E. $\times 1,700$

(Fig. 15). Some amoebae contain small round haematophyl bodies. No clear ectoplasmic layer was observed.

The nucleus measures usually about 2 to 2.5 μ across. It consists of a large, central spherical karyosome, surrounded by a clear zone, which is in turn encircled by a delicate nuclear membrane (Fig. 20). The karyosome stains deeply with Ehrlich's acid haematoxylin, and still more distinctly with iron haematoxylin. Its diameter is usually about 1 μ ; the occasional much larger forms may perhaps be predivisinal or degenerate. Sometimes the karyosome is indented (Fig. 20 C, D) this may represent a stage in division. Occasional amoebae contained two nuclei (Fig. 20 A, B)—evidence of their rapid multiplication.

No forms resembling cysts were recognized.

The problem which immediately arises concerns the identity of the amoeba which has caused such widespread lesions. The only amoeba known to invade human tissue is *Entamoeba histolytica*. Yet the nuclear structure of the present organism is quite different from that characteristic of the genus *Entamoeba*.

A definite nuclear membrane, on the inner surface of which the bulk of the chromatin of the nucleus is distributed in the form of granules—a comparatively small karyosome (WENTON, 1926). On the other hand, the large central, spherical karyosome and delicate nuclear membrane are like those of *Iodamoeba*. The size of the trophozoites—3 to 12 μ , with an average of 6 to 8 μ , in tissue sections—is also consistent with that reported for *I. bütschlii* in faecal smears—5 to 20 μ , usually 9 to 13 μ (WENTON, 1926). (It is regretted that no adequate examination of the faeces was made and that there is not available the additional information that would have accrued from a study of cysts.)

In its behaviour in the tissues, however it resembles *E. histolytica*. It is actively invasive. In fact, the distribution of lesions would be unusually extensive, even for *E. histolytica*. Histolytic action is well demonstrated in the brain sections, and here and elsewhere red cells are being ingested. In the intestinal ulcers (as with *E. histolytica*) amoebae are to be found most frequently just beyond the area of cellular reaction. Although there is much leucocytic infiltration in most tissues in the present case—a feature not characteristic of entamoebiasis—this may well be due to secondary infection.

After examining material from this case, Dr C. M. WENTON was good enough to comment as follows —

"I think it can with safety be concluded that the amoeba is not *E. histolytica*. There are two human amoebae which have some resemblance to the one you have found. I refer to free forms of *Endolimax nana* and *Iodamoeba bütschlii*. Both have centrally placed karyosomes, but that of *E. nana* is not uniformly rounded. It is distinctly polymorphic, and I do not think that this feature would not be shown by so many amoebae as occur in the sections. If we exclude *E. nana* as I think we are bound to do, we are left with *I. bütschlii* the resemblance to which is really very

close Dr HOARE and I have compared free forms of this amoeba as seen in iron-haematoxylin stained faecal films with similarly stained sections which were cut from the blocks you sent, and we both agree that the similarity amounts almost, if not entirely, to complete identity

"If this conclusion is not correct, then one will have to assume that your amoeba is quite new. This would hardly seem justifiable as we have no knowledge of the presence of such a new amoeba in the intestine of your case. If the *I bütschli* view is accepted, then it has to be admitted that the invasion which has occurred is unique for there is as far as I am aware no previous record of this amoebae invading the tissues

"It might be urged that this is an instance of postmortem or immediate antemortem invasion, but the infection is so widespread in the body, and the number of amoebae so great, that some considerable time must have been occupied in the extensive multiplication which has taken place in the tissues after invasion had occurred

"To sum up, therefore, it seems that the most reasonable conclusion is that the case is an instance of unusual invasion of the various organs by an amoeba, presumably one from the intestine, and that structurally this amoeba bears the closest resemblance to free forms of *Iodamoeba bütschli* "

The hitherto non-pathogenic record of *I bütschli* is indeed an objection to a suggestion that it may have been responsible for generalized infection. Yet one remembers that in a proportion (which is variously estimated by different observers) of patients harbouring *E histolytica* in the intestine, there is no apparent invasion of tissues. On this, STRONG (1942) comments

"Whether or not *E histolytica* will produce intestinal lesions in an individual is probably dependent to some extent upon the environment which the protozoa find in the intestine. The hydrogen ion concentration of the intestinal contents and bacterial flora, the previous existence of intestinal lesions, the resistance of the host, and possibly the presence of specific or non-specific substances in the blood, are all factors which may influence at different times the pathogenic action of the amoebae "

NAUSS and RAPPAPORT (1940) demonstrated that irritation of the intestine by croton oil or certain bacteria aided penetration by *E histolytica* of the colonic mucosa in cats. A further factor to be considered is that different strains may differ in their pathogenicity

By analogy with such variation in the behaviour of *E histolytica*, it is conceivable that a set of circumstances might arise which would enable *I bütschli*, ordinarily not pathogenic, to invade tissues. Of the influencing factors mentioned above, an intestinal lesion (dysentery) was present in this case, and it is likely that the host's general resistance was much reduced

I have not been able to find any reference in the literature to the phagocytosis of red blood cells by *I bütschli*. There is, however, ample precedent for a non-pathogenic amoeba doing so. DOBELL (1936), for instance, records that some strains of *Entamoeba coli* avidly ingest red blood cells, an observation confirmed by TYZZER and GEIMAN (1938)

On the whole it seems more likely as suggested by WENTON that the causative amoeba in this case is *I. bütschlii* than that it is an entirely new human pathogen.

DISCUSSION

There seems no reason to doubt that this case represented primarily an infection of the intestinal tract from which the amoebae spread to the other organs affected.

The invasion of vessels—arterioles, venules and lymphatics—in the various lesions readily explains the widespread metastases as well as the areas of necrosis and haemorrhage. An interesting observation was the massing of amoebae around arterioles, which was especially noteworthy in the sections of brain, right lung and mesenteric and gastric lymph nodes.

It is curious that the liver showed no apparent evidence of amoebiasis, for with *E. histolytica* it is the most frequent site of metastatic infection. Invasion of intestinal venules was observed (Figs. 7 and 17) and by this route many amoebae must have reached the liver.

The multiple amoebic ulceration of the stomach calls for comment. It is generally accepted that amoebic infection is spread by ingestion of cysts, any swallowed trophozoites being destroyed by the gastric secretion. In this case it is suggested that the number of amoebae in the patient's stools was large, that the sanitary conditions under which he was living before capture were primitive and his general resistance was low because of malaria, dysentery and malnutrition, that his gastric secretion failed, and that the combination of these conditions permitted numerous trophozoites to be ingested and to invade the gastric mucosa.

SUMMARY

A Japanese soldier aged 22, captured in New Guinea, died 7 weeks later from generalized amoebiasis.

There were amoebic ulcers in stomach, small intestine caecum, ascending and transverse colon, as well as metastatic amoebic foci in gastric and mesenteric lymph nodes, both lungs and the brain.

The causative amoeba was either *Iodamoeba bütschlii* or one closely resembling it.

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REMARKS OF WELCOME TO THE
ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE*
WASHINGTON, D C , 14TH MAY, 1948

BY

M E BARNES, M D ,

*Professor and Head, Department of Hygiene and Preventive Medicine, College of
Medicine, State University of Iowa, Iowa City, Iowa*

It is indeed a signal honor to have been asked by the local secretaries of North and Central America to address a few words of welcome to the guests present on this happy occasion. The assignment is made easier both for the speaker and the guests by the instructions of Dr A J WALKER. According to my understanding of his instructions, I am to follow rigidly the three rules which were enunciated many years ago for the guidance of speakers.

The first rule is—"Be sincere". In this instance it is easy to obey this rule because all of you from areas other than North and Central America are the invited guests of those resident in the Americas. This fact in itself attests realistically the sincerity of our welcome. Incidentally, of the total membership of the Royal Society of Tropical Medicine and Hygiene approximately one in seven resides in North and Central America.

But before continuing, I must remind myself and the audience of the second rule for speakers, which is—"Be brief".

Briefly, then, let me point out that many of those serving as hosts today are members also of another society, the American Society of Tropical Medicine. Until FAUST's article (1944), summarizing its history, I had always assumed

* Made by Dr BARNES at a luncheon party given by U S A Fellows of the Royal Society of Tropical Medicine and Hygiene, to Fellows from overseas attending the Fourth Congresses of Tropical Medicine and Malaria.

that the American Society was the younger of the two. The contrary is true. The American Society was founded in 1903, whereas the Royal Society had its beginnings in 1907. I mention this to point out certain matters of common interest to both societies.

First, Sir PATRICK MASON wielded a powerful influence in both organizations. He was included in the first list of honorary members elected by the American Society in December 1903. His influence in the Royal Society is well known to all.

Second, both societies were organized in response to medical interests of an urgent nature. The American Society was organized in response to the urgent interest in tropical diseases resulting from newly assumed responsibilities in the Philippines and the West Indies, and the agitation for the construction of the Panama Canal. Although Britain had long faced the problems of tropical medicine, never was any empire since the dawn of history faced with so serious and catastrophic a problem in public health as was involved in the plague epidemic in India which in 1907 was mounting toward its peak.

Interest in tropical medicine at that time received a very great impetus, also from the discovery of the role of mosquitoes in the transmission of malaria and of yellow fever.

Today we have just emerged from a world war in which interest in tropical diseases has been forced upon us in a manner and to a degree never before experienced.

In this connection, it is pertinent to point out two points of striking importance which are evident in this congress which we are attending.

One is the extraordinary optimism and confidence with which we face tropical diseases today. This is in marked contrast to the situation which existed when our Society was founded. Four examples may be cited.

(a) MALARIA.

The papers and discussions at this congress display an amazing confidence in our ability to control malaria. As Dr PAUL RUSSELL has remarked, the application of DDT to the control of malaria appears to be the greatest thing that has happened in that field since the discovery of the role of mosquitoes in its transmission. Even though it may not be applied at all times with efficiency this measure can be brought within the financial reach of the humblest villager in the most malaria-ridden areas of the world. This can be said of no other antimalaria measure which has been suggested heretofore.

(b) DISEASES CARRIED BY *Phlebotomus*.

The discovery that the organisms causing Oroya fever and kala azar were conveyed by *Phlebotomus* seemed at the time to make the control of these

Personal communication.

diseases even more hopeless than before we had this knowledge. Who could have believed five years ago that DDT could accomplish the local eradication of *Phlebotomus* from adobe or stone village houses which has been reported in recent journals by HERTIG (1948) and others

(c) PLAGUE

Plague, likewise, is feeling the effects of our new weapons of attack and of defense. Describing its control in Haifa by DDT, sulfadiazine and streptomycin, POLLOCK (1948) said that these results "make us hope that the world has seen the last widespread plague epidemic." These are brave words indeed to apply to a disease justly classified as one of the greatest killers of mankind throughout the ages of the past. I was in India during the period 1905 to 1908, when the plague epidemic reached its maximal prevalence in the Punjab. At that time the health authorities were both helpless and hopeless in their efforts to combat and control that dreaded disease. The present-day attitude shows a marked contrast. The optimistic hopes of Dr. POLLOCK have been repeated over and over by the delegates attending this congress.

(d) LEPROSY

Leprosy, which has always been a source of disappointment, is now perhaps in the process of being reclassified. At any rate MUIR (1948), who is not a man to be swept off of his feet, although remaining non-committal as to the fact of cure, has pointed out some startling results from sulphone therapy. He says that there "seems no doubt that in most cases the bacilli in the skin and mucous membranes of the nose diminish till they become difficult or impossible to find. The presumption is that such cases become less capable of transmitting the infection."

A second significant feature of these sessions is the emphasis being placed upon hygiene in the tropics. Man, originally a tropical animal, never has succeeded in subduing the tropics. Temperature, humidity, luxuriant vegetation and disease heretofore have limited his efforts in the tropics. Now, the pressure of populations and the world's need for food will ere long compel us to subdue the tropics. With our new weapons, our hopes of subduing tropical diseases are brighter than ever before. With the new chemical methods of controlling weeds and vegetable growth, the possibility exists of reducing the man-hours needed in tropical agriculture. STRODE (1948) has pointed out that by air conditioning of homes and reversing the habits of work to the cooler parts of the day or to the night, some of the thermal problems can be overcome. Renewed research into the physiologic aspects of nutrition and climatic adjustments will yield more new knowledge. It would appear, therefore, that we should now give greater stress to the last two words in the name of our Society, namely, The Royal Society of Tropical Medicine and Hygiene.

In these remarks, I have followed the first two rules mentioned, namely: I have been sincere, and I have been brief. I now propose to follow the third rule, which is "Be seated."

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CORRESPONDENCE.

A NOTE ON THE VALUE OF FOLIC ACID IN THE TREATMENT OF MACROCYTIC ANAEMIA IN ASSAM TEA GARDEN LABOURERS *

To the Editor, *TRANSACTIONS of the Royal Society of Tropical Medicine and Hygiene*

SIR,

Folic acid is now well established as a successful treatment for megaloblastic anaemia, and there is nothing original in this brief report. However, the life-saving properties of folic acid have been so striking that they may be of general interest.

In pernicious anaemia, liver extracts are still the drugs of choice while folic acid is a valuable supplementary or reserve treatment. In macrocytic anaemia of pregnancy and nutritional anaemia the position is reversed, and we have obtained results with folic acid more swiftly and completely than had previously been possible with any liver extract preparation.

The tea garden labourers of Assam are predominantly rice eaters, supplementing their diet with occasional vegetables but rarely with meat. NAPIER (1936)† found the average haemoglobin of apparently healthy labourers to be 11 to 12 grammes per 100 c c, though in the post-war years in a small series of 50 healthy workers we found the average to be 10 grammes per 100 c c, using Sahl's method.

Many of the cases recorded below had blood pictures barely compatible with life. They form only a section of almost 100 cases, most of whom were not followed sufficiently accurately for official recording. The dose of folic acid was 5 mg orally, b d for 10 days in all cases.

* All the haematological data have been supplied by Dr J N BATASYAL, D T M (CALCUTTA), without whom this note could not have been written.
† Haematological Technique (NAPIER and SEN GUPTA)

Clinically they presented symptoms of lassitude and weakness and showed gross oedema, pallor of the tongue and mucosae, tachycardia, cardiac dilatation with haemic murmurs, and in 12 of the 20 cases an irregular unexplained pyrexia up to 101 F. The response to folic acid was rapid. Subjectively many cases were better on the third day. Oedema was reduced with remarkable speed, being complete by the fourteenth day in all cases and often earlier. This is presumably related to the improved state of the cardiac muscle. An interesting feature was the control of irregular pyrexia without any additional treatment. This occurred between the third and fifth days. Continued improvement up to 21 days after the tenth day of treatment was noted.

Only once have we found treatment to fail and in this case there was a megaloblastic blood picture with bleeding from the gums and petechiae in the skin. The patient appeared gravely ill on admission, but survived 16 days, so that it is possible that larger doses would have succeeded. It was not possible at that time to give parenteral folic acid.

Cases 1 to 5 had already received injections of crude liver extract up to 60 c.c. before folic acid was given.

Case No.	Type	Hb. Grammes per 100 c.c.	R.B.C. per c.mm.	M.C.V.	Interval between blood counts.	Hb.	R.B.C.	M.C.V.
					Days.			
1	P	3.8	770 000	143	16	9.8	2,450 000	85
2	P	3.8	850 000	170	30	9.0	4,030,000	85
3	P	3.8	950 000	90	28	7.0	3,800,000	99
4	P	5.0	1,300 000	120	28	9.0	3,370 000	90
5	N.C.	4.0	1,670 000	108	20	10.9	2,310 000	102
6	P	4.9	2,610 000	163	30	9.8	3,180 000	90
7	P	3.0	900 000	120	30	55 per cent. Talquist		
8	P	3.9	800 000	140	28	55		
9	N.M.	3.5	910 000	118	21	58		
10	N.C.	4.0	940 000	122	14	55		
11	P	4.0	980 000	100	21	55		
12	P	3.5	840,000	172	28	85		
13	N.C.	4.0	990 000	120	16	85		
14	P	9.5	1 940 000	109	14	86		
15	P	8.5	1 370 000	149	18	60		
16	P	3.8	1 110 000	85	19	83		
17	P	4.9	1,510,000	160	19	88		
18	P	4.0	1,219 000	194	30	58		
19	N.C.	2.5	776 000	123	30	85		
20	P	4.0	1 230 000	112	19	95		

P — Pregnancy anaemia N.M. — Nutritional anaemia in male N.C. — Nutritional anaemia in child
55 per cent. Hb. Talquist roughly corresponds to 9 to 9 grammes Hb. per 100 c.c.

SUMMARY

The results of treatment with folic acid in 20 severe cases of pregnancy or nutritional anaemia are recorded. As judged by European standards, the final results are anything but normal, but they do demonstrate the life-saving properties of folic acid which should be regarded as a specific drug in this difficult and often fatal condition.

I am, etc ,

P H BIRKS,
Medical Officer

Medical Department,
Beesakopie, Doom Dooma,
Upper Assam
26th March, 1948

HATCHING SPEED OF SCHISTOSOME MIRACIDIA

SIR,

We have not found any reference to the speed with which the miracidia of *Schistosoma haematobium* can emerge from their egg shells. Unless the miracidium becomes entangled by its ciliary hairs in the rent in the shell, it would appear to hatch with great speed.

It is common experience in the observation of the hatching process on the microscope stage, to look away for an instant to rest the eyes, and find on returning to the eyepiece that all that remains is an empty shell with no miracidium in view.

In preparing material for a film on Schistosomiasis, the opportunity was taken to study the individual "frames" of cine film exposed during the act of hatching. We found a frame where the egg was intact, and in the next frame the egg shell was split and empty and the miracidium already a short distance away from the shell. The images in the two frames were quite clearly defined so that the act of emergence must have coincided with the instant of time when the film was not exposed to the light transmitted through the microscope slide.

The film was being exposed at the rate of 24 frames per second, so it would appear that the hatching took place within the 1/48th of a second while the shutter was closed. The temperature at which the hatching took place was room temperature, about 72° Fahrenheit.

We are, etc ,

FRANK A GOODLIFFE,
D M BLAIR, M B , D P H

Salisbury,
Southern Rhodesia

CORRIGENDA

Vol. 42, No. 1 July 1948

R. H. GIRDWOOD. Anaemia and malarina in Indian troops on active service

Page 82 (Footnote) For haemoglobin " read haemoglobin,
for taking 100 per cent. as 10.8 G per 100 ml."
read taking 100 per cent. as 14.8 G per 100 ml.
j

[The previous number of these Transactions, Vol 42 No 2
was published on September 27th 1948]

TRANSACTIONS
OF THE
ROYAL SOCIETY OF TROPICAL MEDICINE
AND HYGIENE

VOL 42 No 3 NOVEMBER, 1948

ORDINARY MEETING
of the Society held at
Manson House, 26, Portland Place, London, W ,
on
Thursday, 21st October, at 7 30 p m

THE PRESIDENT,
SIR PHILIP MANSON-BAIR, C M G , D S O , F R C P
in the Chair

PAPERS

THE EPIDEMIOLOGY OF FUNGUS DISEASES

by

JAMES I DUNCAN

Director of Medical Mycology, London School of Hygiene and Tropical Medicine

There is a marked divergence of opinion on the importance of fungus diseases in the British Colonial Empire. Some people, impressed no doubt by the extreme paucity of the literature of medical mycology from British colonial sources, believe that fungus diseases in the tropical colonies are rare or trivial, while others, who seem to see a fungus disease in every repulsive disorder of the skin from which a crop of contaminating moulds can be cultivated, consider medical mycology to be essentially an interest of tropical medicine. In fact, little is known of the fungus diseases of the colonial empire, whether they be common or rare, because little attention has been paid to them. By far the largest numbers of fungus infections have been found in countries with subtropical or temperate climates, but that, in great measure,

may be attributed to the better laboratory facilities, the specially trained personnel, and, particularly the greater interest taken in fungus diseases in those countries. It cannot too strongly be stressed that some of the more serious systemic mycoses may elude diagnosis unless they are sought for specially.

THE DERMATOPHYTOSES.

Amongst pathogenic fungi which are transmitted more or less directly from animal host to animal host, the best example is the dermatophytes or ring-worm fungi. These fungi have established themselves as parasites on the keratinized tissues of the skin and its appendages and they have developed a high degree of host specialism, infecting chiefly the young of the host species. There is a small group of dermatophytes which are parasites on man only being transmitted from child to child and some of these, e.g. *Microsporum audouinii*, live in a state of almost perfect parasite host equilibrium, combining high infectivity with low pathogenicity the host even aiding the propagation of the parasite on to new hosts. The parasitic association however comes to an end spontaneously during adolescence.

M. audouinii, which was formerly found chiefly in North-Western Europe, has now established itself in North America and is making its appearance in other parts of the world. Recent epidemics of tinea capitis due to *M. audouinii*, involving some thousands of cases in the United States and hundreds in Britain, are attributable chiefly to relaxation of the normal pre-war measures of treatment and control and to the migrations of children under war time conditions, but a factor which may also play a part is reduced nutrition which may render the potential host more susceptible to infection and favour the occurrence of unusual types of lesion, such as tinea circinata on the glabrous skin and infection of young adults by *M. audouinii*. DOWK, quoted by NICKERSON (1947), observed an increased susceptibility to epiphytic diseases in prisoners of war on a reduced diet, in the hands of the Japanese.

Microsporum canis (syn. *M. felisum*) the common *Microsporum* of dogs and cats, is a frequent cause of tinea capitis and tinea corporis on children and occasionally on adults. The species has a much wider geographical distribution than *M. audouinii* but it does not cause large epidemics. Infection, in the first instance, is transmitted from cat or dog to child, and thereafter it may be propagated through a limited number of transfers, probably four to six, from child to child before the infection dies out. Although an alien parasite, it is well adapted to its human host and the lesion on the scalp is usually indistinguishable from that caused by *M. audouinii*. *M. canis* is, however, slightly more pathogenic and less infective for children than *M. audouinii* and the infection on the scalp responds more readily to topical treatment, but the control of the disease is rendered more difficult by the existence of animal hosts. Another parasite of animals which not infrequently infects man is *Trichophyton discoides* a cause of cattle ringworm in many countries. This

species, which belongs to Sabouraud's *Trichophyton-microporum* group of *Trichophyton*, is almost invariably transmitted directly from the infected animal, usually a cat, to man. On man it is an ill-adapted parasite showing low infectivity and usually high pathogenicity, sometimes causing suppurative lesions (kerion, syccosis or agminate folliculitis), which bring the infection to an end by the spontaneous discharge of the infected hairs. Transmission of the infection from man to man is probably very rare. Selective infectivity of a high order would therefore seem to be characteristic of the well-adapted parasite while pathogenicity suggests the ill-adapted alien or, in other words, pathogenicity tends to diminish as selective infectivity increases.

Trichophyton mentagrophytes (many synonyms) belonging to Sabouraud's *Trichophyton-microporum* group of *Trichophyton* is a very widely distributed parasite found on rodents, horses, cat and sometime on dog and cattle and it is probably the commonest *Trichophyton* found on man. Infection is usually by direct transfer from an infected animal or hide or from spores of the parasitic stage shed in stables, etc. even the possibility of rare infections from a saprophytic growth of the fungus on stable refuse cannot be excluded. The lesions on children and adults may be simple or suppurative. In Britain there is little tendency for further transmission from man to man but in warm climates, e.g., Bombay and Singapore, outbreaks of body ringworm on white troops have been caused by this species, the infection, apparently, being transmitted from man to man by exchange of shirts, etc. *T. mentagrophytes* including the variety *T. interdigitale*, is also the most frequent cause of tinea pedis, an infection transmitted from man to man.

TINEA PEDIS

Tinea pedis, also called athlete's foot Hongkong foot, etc., is caused mainly by three species of ringworm fungi: *Trichophyton mentagrophytes* (syn. *T. interdigitale* etc.), *T. rubrum* (syn. *T. purpureum* *Epidermophyton rubrum*, etc.), and *Epidermophyton floccosum* (syn. *T. cruris*), which are transmitted by transfer in scales from the feet of the infected to the interdigital clefts, especially the fourth cleft, of the feet of others. As *T. mentagrophytes* can vegetate on the shiny surface of wet wood, the possibility of rare infections by the saprophytic form of this species may be admitted. Conditions which favour the transmission of the disease are the congregation of people in schools, universities, military camps and barracks, on shipboard and in certain industries, with some easy means of transfer of foot infections, such as the use of communal swimming and shower baths and the exchange of socks, gym shoes, towels, etc., aided by factors which induce a hot and damp state of the feet, such as athletic games, physical training, military exercises and coal mining. Domestic infections may be contracted in tropical bathrooms. Indeed, it may be said that the dissemination of tinea pedis is aided by the cultivation of hygienic practices.

There is little doubt that the two world wars have played an important

part in the spread of *tinca pedis* in western countries. The disease was almost unnoticed before 1914 but in the decade following the first world war extensive outbreaks of athlete's foot occurred in educational establishments in the United States and Britain, and also in athletic clubs and in certain industries. *Trichophyton rubrum*, a native of the Far East, has for some years past been spreading over the western countries, and the incidence of this species on athlete's foot in the United States and especially in Britain, has markedly increased since the recent war—no doubt by importations from S.E.A.C. and other Far Eastern war theatres. In deciding on the probable time of infection, it should be remembered that *tinca pedis* may sometimes be dormant or subclinical in cold or temperate climates but manifest itself as a severe and often crippling disease under tropical conditions.

Tinea cruris is often associated with *tinca pedis* and is caused by the same species of fungi.

THE SYSTEMIC MYCOSES.

The more important of the systemic mycoses are caused by fungi which are dimorphic in the sense that their parasitic form, seen in the lesion, differs strikingly from the natural saprophytic form. In saprophytic life the fungus vegetates in soil or on some vegetable substratum as a mycelial growth often bearing an abundance of spores which, no doubt, constitute the chief infective element, especially in air-borne infections. In parasitic life, however, the fungus develops a simple form, frequently unicellular and yeast-like, well adapted for life in animal tissues and for rapid multiplication, invasion and defence. In contrast to the ringworms, the systemic mycoses caused by dimorphic fungi are not transmitted directly from man to man, except in rare accidental instances associated with autopsies or surgical operations, etc. for the parasitic form of the fungus is ill-suited for transmission, and outside the body it dies or under appropriate conditions, it reverts to the mycelial form. It is true, of all the dimorphic fungi, that a simple form resembling the parasitic form can be developed under special conditions in artificial culture, but it is improbable that this occurs in nature. Infection, therefore, is caused by the introduction into the body of spores or other elements of the saprophytic form of the fungi vegetating in man's environment, and the migrant human carrier plays little or no part in the epidemiology of these mycoses. Indeed, the factors on which the natural saprophytic vegetation of the fungus depends may largely determine the geographical distribution of the disease. Some mycoses, e.g., histoplasmosis and sporotrichosis are widely distributed in the world, while others, e.g. the North and South American types of blastomycosis, are indigenous to certain large geographical areas and still others e.g., coccidioidomycosis, are endemic in strictly localized areas.

The systemic mycoses offer an interesting problem in epidemiology for the phenomenon of dimorphism and the ready adaptability of the saprophytic

JAMES T. DUNCAN

fungus to parasitic life seem to indicate a well-established parasitic habit, which is difficult to explain in connection with diseases believed to be rare and very sporadic, unless it be accepted that subclinical or unrecognized infections occur in man (there is evidence of such infections), or that an animal reservoir of the disease exists. Alternatively, it may be contended that dimorphism, upon which pathogenicity depends, is a property inherent in these fungi, a convenient explanation of the sporadic character of an apparently highly fatal infection. It is worth mentioning that dimorphism is common amongst the *Entomophthorales*, which are parasites in arthropods.

COCCIDIOIDOMYCOSIS

While little is yet known of the source and the mode of infection in many of the systemic mycoses, much light has been directed recently on the epidemiology of coccidioidomycosis. This disease, which bears some resemblance to tuberculosis in its clinical and pathological features, is endemic in desert areas of the South-Western United States and the Chirico region of South America. The causative fungus, *Coccidioides immitis* Rixford and Gilchrist, 1896, vegetates in the rather calcareous desert soil during the wet season, producing a great abundance of chlamydospores measuring about 3 by 4 μ . In the succeeding dry season these spores, dispersed and carried with the wind-borne desert dust, may be inhaled by man and cause, after an incubation period averaging 14 days, primary coccidioidomycosis, a disease long known in the endemic areas under the names of "desert rheumatism" and "Valley fever." In a small proportion of cases, possibly 1 in 400, the primary disease progresses to the better known, grave coccidioidal granuloma stage. Infection may also occur, although rarely, through wounds of the skin. EMMONS and ASHBURN (1942), who identified the disease in significant numbers of two genera of desert rodents, the "pocket mice," *Perognathus baileyi*, *P. penicillatus* and *P. intermedius*, and the "kangaroo rat," *Dipodomys merriami*, suggest that these animals play a necessary part in maintaining the fungus in the soil, an observation which would serve to explain the restricted distribution of coccidioidomycosis and perhaps also the dimorphic character of *C. immitis* and its ready adaptability to parasitic life. The role of an animal reservoir of a fungus disease which is not transmitted directly from host to host is difficult to assess, but it may be to preserve the parasitic habit in a saprophytic fungus.

The discovery that a mild and frequently subclinical form of coccidioidomycosis is widespread in the endemic areas in the United States where for nearly half a century it had been recognized only in its relatively rare coccidioidal granuloma form, suggests the need for a renewed investigation of the disease in other parts of the world, especially in South America, where, since the original discovery of coccidioidal granuloma by WERNICKE and POSADAS in 1892, less than a dozen cases have been reported in the literature, and in Italy where, according to REDAELLI and CIFERRI (1934), two suspected cases

were found. The disease has not yet been reported from the desert areas of the old world tropics.

SPOROTRICHOSIS.

Sporotrichosis is a widely distributed but relatively rare sporadic disease characterized by subcutaneous and cutaneous gummatous nodules and ulcers, often with an associated mycotic lymphangitis. The causative fungus, *Sporotrichum schenckii* (Hektoen and Perkins, 1900) Matruchot, 1910 has been found on several kinds of plants and the infection of man has been attributed to the inoculation of the fungus with scratches or penetrating wounds of the skin caused by barberry or rose spines, or contamination of open wounds by sphagnum moss on which the fungus had been vegetating. *S. schenckii* can also vegetate freely on sound timber of several kinds and, given favourable epidemiological conditions, it may cause large outbreaks of disease, as was shown recently in the Witwatersrand gold mines (1947) when 2,825 mine workers were infected by the fungus vegetating on untreated mine timbers. Cuts or abrasions of the skin were found to be an important factor in these infections, and it is noteworthy that despite the concentration of the fungus, the confined space and the existence of ventilating air currents, infection by the pulmonary route, apparently did not occur. It is not known if the fungus on the mine timbers was introduced by a miner suffering from sporotrichosis or if the timber acquired the fungus in the timberyard or even in the forest.

CHROMOBLASTOMYCOSIS.

Chromoblastomycosis (dermatitis verrucosa "mossy foot, etc.) is a chronic mycosis affecting the skin and subcutaneous tissues, chiefly on the extremities and exposed parts of the body characterized by the local development of papular verrucose and sometimes ulcerating lesions the most typical being the cauliflower-like, scumpeunculated growths from which the name "mossy foot" is derived. The disease, although sporadic and rare, is widely distributed in the New World, and it is also found in North, South and East Africa, Malaya and the Far East, Australia and Russia. The causative fungi, which are seen in the lesion as little rounded, sometimes septate bodies of deep brown colour measuring about 8 to 10 μ in diameter belong to the genus *Phialophora* (syn. *Fonsecaea*) and the species *P. verrucosa*, *P. pedrosoi* and *P. compacta*. These fungi, in saprophytic life, vegetate as mycelial colonies of an olive green to blackish colour and are related to common moulds found on dead vegetable substrate. A common saprophyte, *Cadophora arvensis* which causes discoloration of wood pulp is believed by COVANT (1937) to be identical with *Phialophora verrucosa*. Despite the slow evolution of the lesion, it has been possible in several cases to obtain a history suggesting infection through an injury to the skin caused by some vegetable material.

JAMES T DUNCAN

NORTH AMERICAN BLASTOMYCOSIS

North American blastomycosis (Gilchrist's disease) is a subacute or chronic mycosis characterized by papillomatous, papulo-ulcerative, gummatous or suppurative lesions of the skin and subcutaneous tissues, with a primary but frequently transient pulmonary infection in a majority of the cases, and occasionally the development of a generalized, fatal disease. This mycosis is indigenous in North America, where it is widely distributed in the United States, the highest incidence being in the region of the Mississippi basin, especially its northern and southern extremities. In spite of reports of the disease from other parts of the world, the literature contains only two well authenticated records of the infection occurring outside North America, in one in London and in the other, reported by BRODY (1947), in France. It may be significant that in both cases the patient had been handling materials imported from the United States. Nothing is known of the source of the infection or of the natural saprophytic life of the causative fungus, *Blastomyces dermatitidis* Gilchrist and Stokes. The age and the occupation of patients shed little light on the epidemiology, but the disease appears to be about nine times as frequent in men as in women and it has been found in the dog. There is no doubt that, while infection sometimes takes place by inoculation through the skin, in many cases, estimated by MARTIN and SMITH (1939) at 50 per cent., and by SKINNER *et al* (1947) at 95 per cent., the earliest symptoms are referable to the lungs, suggesting infection by inhalation.

SOUTH AMERICAN BLASTOMYCOSIS

South American blastomycosis, caused by *Paracoccidioides brasiliensis* (*Blastomyces brasiliensis*) is known only in South America, where it is widely distributed, but the greatest number of cases has been found in Brazil. The epidemiology of the disease is obscure but the infection seems to be most frequent amongst agricultural workers and the almost constant occurrence of early lesions on the oral and adjacent mucosae may indicate infection by ingestion. In a few reported cases the disease was confined to the ano-rectal region and infection appeared to have been caused by using, for the toilet of the anus, leaves or other raw vegetable materials on which the fungus may have been living saprophytically. A possible indication of the natural habitat of the fungus

HISTOPLASMOSIS

In histoplasmosis the phagocytes of the reticulo-endothelial system are invaded by the small, capsulated, yeast-like parasitic form of *Histoplasma capsulatum* Darling, 1906, causing local hyperplasia of the reticulo-endothelial cells with the development of diffuse or nodular lesions in various organs, sometimes with necrosis, haemorrhage or fibrosis. This disease, of almost protean

symptomatology at present occupies the focus of epidemiological interest. Histoplasmosis was discovered by DARLING, in Panama, who described three fatal cases in 1905-06. Since then no other human case has been identified in Panama, although a canine infection was found there recently. The next report of the disease was in 1928, 20 years after DARLING's discovery when a case was diagnosed in Minnesota, and since then it has been identified in many parts of the world, including England, but in some countries only a single, although fatal case has been found. By far the greatest number of cases has been reported in the United States, especially in the central-eastern part. The epidemiology is obscure and the disease seems to have no special relation to occupation, sex or age, although it is interesting to record that a considerable proportion of the patients have been very young children, the youngest being only 7 weeks old. Histoplasmosis has been found in ten instances in the dog, and FARROW *et al.* (1947) have isolated *H. capsulatum* from wild rats. Nothing is known of the natural saprophytic life of the fungus or the mode of infection, but the frequent occurrence of pulmonary lesions seems to indicate infection by inhalation, and, in this connection, the similarity of one of the spore forms of *H. capsulatum* and of *Blastomyces dermatitidis* is interesting.

Following the successful application of mass radiography of the lungs and tests of specific dermal sensitivity to coccidioidin in the epidemiological study of coccidioidomycosis, similar methods have been applied in large-scale surveys for undetected histoplasmosis, by PALMER (1945-1946), CHRISTIE and PETERSON (1945-1946), FURCOLOW, HIGH and ALLEN (1946), FENSTER and FURCOLOW (1947), ZWERLING and PALMER (1947), HIGH, ZWERLING and FURCOLOW (1947), and others in the United States. Commencing with the discovery in parts of the central-eastern area of the country of numbers of young people with healed pulmonary lesions who were tuberculin-negative but histoplasmin positive, a survey of accessible groups of the population revealed a very significant geographical distribution of reactors to the histoplasmin skin sensitivity test. On this basis a close study was made of reactors by FURCOLOW, MANTZ and LEWIS (1947), and BRYNELL and FURCOLOW (1948), ending in an intensive clinical and mycological examination of all who showed signs of active disease, with the result that several cases of unsuspected histoplasmosis were brought to light. There can be little doubt that subclinical or unrecognized cases of histoplasmosis exist and may indeed, be numerous in certain parts of the United States and possibly in other countries, not excluding our own colonies, in which indigenous histoplasmosis exists.

TORULOSIS

Torulosis, sometimes called European blastomycosis, which affects chiefly the brain and its meninges, the lung and sometimes other organs, has a world wide distribution, but is a relatively rare disease in all countries. The greatest number of cases has been reported from the United States, but the highest

number in proportion to the population from Australia. The causative fungus, *Cryptococcus neoformans* (Sanfelice) Vuill. has been found in milk and milk products, in transfusion plasma, on the skin and on the buccal mucosa of man, on preserved fruits and other foods. The path of infection has not been proved but the nasopharyngeal mucosa and the tonsil are possible portals, although inhalation of the dry, resistant yeasts may prove to be the principal mode of infection.

MONILIASIS

Moniliasis has a world-wide distribution and the causative fungi, *Candida albicans* and *Candida tropicalis*, are fairly common inhabitants of the healthy human mouth, intestine and vagina. TALICE (1932) has reported an apparent *C. albicans* in the alimentary canal of hedgehogs, and species of *Candida*, not studied for diagnosis, have been found in dogs, cats, goats and fowls. Infection of the mucosae, the skin and sometimes the deeper organs in man, is usually autogenous, but transmission from child to child, often with exaltation of virulence, occurs in nursery and creche outbreaks of thrush.

ACTINOMYCOSIS

In actinomycosis, infection by the anaerobic species *Actinomyces israeli* (syn. *A. bovis*) is probably invariably autogenous, the fungus being a common inhabitant of the human mouth and, apparently, incapable of natural saprophytic survival outside the body.

MYCETOMA

Mycetoma is chiefly a tropical disease, although it also occurs in subtropical and even in temperate climates. It is caused by many different species of fungi which are presumed to live naturally as saprophytes on vegetable substrata. Infection, through the skin, is caused by the mechanical introduction of the fungus on some penetrating foreign body which may play a subsequent role in establishing the disease, or it may be introduced through an open wound or ulcer. These fungi are not dimorphic and can only vegetate in animal tissues as a mycelial colony or "grain," causing a localized disease with no tendency to metastatic spread.

I have given a brief and very sketchy account of only one aspect of medical mycology, but it should be evident that a great deal has yet to be learned of the epidemiology of the systemic mycoses, including even their geographical distribution. Some of these diseases are difficult to detect by the ordinary clinical methods, and they may be overlooked unless an adequate mycological examination is made. A survey of the literature might give the impression that fungus diseases existed chiefly in the United States and a few other countries, but the volume of publications on medical mycology is often largely a reflection of the local interest in the subject and the facilities available to the investigator.

A survey of the fungus diseases in the British colonies is long overdue, and the first step necessary is to provide special training in medical mycology and mycological methods for local bacteriologists and pathologists. One way of meeting this need would be to include medical mycology as an elective subject in the curriculum for the Diploma in Tropical Medicine.

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TREATMENT OF FUNGUS DISEASES OF THE SKIN

BY

I. MUENDE M. B.Sc., M.R.C.P.

Physician and Director of Pathology St. John's Hospital for Diseases of the Skin, London.

Although the study of mycology antedates that of bacteriology by several decades, there has, so far, been no scientific approach to the problem of treatment comparable to that which we have witnessed in the fields of chemotherapy and antibiotics relating to bacterial diseases. In the main, the means which have been employed to eradicate fungus infection of the skin have been of

a physical nature with chemistry playing only a non-specific role. It does not, of course, follow that we have been unsuccessful in treating fungal infections, on the other hand, most of the mycotic infections of the glabrous skin and scalp respond quite successfully to measures employed 30 and more years ago, but in this age when the modes of yesterday are considered antiquated many feel that the therapy of fungus diseases, like its younger sister, bacteriological affections, should acquire a "new look."

Until then it would be best to acquaint ourselves with the factors which influence treatment and the therapeutic measures which have stood the test of time. Of these factors the most important are the tissue which the fungus has attacked, the pathogenicity of the fungus and the source from which it could be conveyed.

The first two are closely interwoven and have a direct bearing on treatment of the individual patient, and the latter on measures to be taken to avoid spread of infection to others, and also re-infection.

The dermatophytes, in their parasitic existence, seek and thrive on keratinized tissue and therefore find the superficial layers of the epidermis, the hairs and nails, excellent soil for their growth, producing, as a result, diseases of varying degrees of severity and persistence. The dermatropic properties of these fungi has been demonstrated by injecting cultures intravenously into guineapigs when, although spores could be found in every organ, they failed to effect any pathological change, but even slight scarification of the skin resulted in the development of local infection and invasion of the hairs. Again, in some cases of *Trichophyton* infection with general malaise, pyrexia and regional adenitis, although fungal elements have been demonstrated in the lymphoid tissue, no signs of local invasion have been detected and everything points to trichophytosis being due to sensitization to a fungal toxin. All the dermatophytes are capable of invading the stratum corneum, but whereas the great majority can also attack hairs, some cannot and these include the species of the genera *Epidermophyton* and *Endodermophyton* and also some ill-defined species such as the *Kaufmann-Wolf* fungus usually considered a *Trichophyton*. If we were to stray further amidst the vague group of fungi we might also include *Microsporon furfur* and the so-called *Microsporon minutissimum*. Even one of the microspora, *Microsporum audouinii*, which seems to have made man its special host, whilst readily capable of invading the scalp hair in children, loses this property at puberty. This has, without sufficient proof, been attributed to a change in surface pH of the scalp developing at puberty.

Treatment of these relatively superficial mycotic infections is, on the whole, not difficult and the measures employed are the use of keratolytics to remove the surface of the stratum corneum, and non-specific fungicides, occasionally used singly but frequently in combination with the former. The commonest keratolytics are salicylic acid and resorcin, but the number of so-called fungicides is legion and their enumeration far beyond the scope of this communication.

One of the oldest fungicides is tincture of iodine, but although efficacious it has been abandoned by most owing to its tendency to give rise to a sensitization dermatitis more troublesome at times than the primary infection. Benzoic acid, a poor fungicide, is still employed with salicylic acid in the form of Whitfield's ointment and in recent years claims have been made for propionic and undecylenic acids and their salts. Although good reports of their value have been forthcoming from some quarters, their efficacy has on the whole proved very disappointing. Chrysarobin and its synthetic analogues have been employed in resistant cases but such preparations should be used with great caution in view of their liability to give rise to severe inflammatory changes. Sodium thiosulphate in the form of a saturated aqueous solution is commonly used, and with success, in the treatment of tinea versicolor due to *Microsporum furfur* but although the response is invariably rapid, the affection is notorious for its tendency to relapse. Whether this is due to the fact, as many believe, that the surface secretion of such patients is particularly suitable to the growth of the organism or that its spores may persist in lanugo follicles out of the range of the medicament, has not yet been ascertained.

Castellani's paint, the active ingredients of which are probably basic fuchsin and phenol, but which also contains boric acid, resorcin and acetone, has been used extensively in various climates and with a fair degree of success. It has the advantage of being capable of penetrating the superficial layer of the epidermis, of desiccating it and finally desquamating the infected tissue. It is therefore frequently resorted to in cases of tinea cruris and particularly the vesicular and bullous *Trichophyton* infections of the feet which do not respond to the usual unguents. Its chief disadvantages, however are that its fungicidal properties deteriorate with time, particularly in warm climates, and that it is impossible to remove the pigment from garments stained by it.

Good results have been claimed for phenyl mercuric nitrate, but whilst acknowledging its value as a fungicide, it is the experience of many that it is occasionally the cause of a dermatitis medicamentosa and is, if used with caution like chrysarobin, best reserved for resistant cases.

Despite our large and varied therapy there are undoubtedly cases which appear to resist all known preparations. The number however is by no means as large as some would be led to believe, for many cases encountered in this category are frequently found to be examples of dermatoses such as inter triginous psoriasis, seborrhoeic dermatitis and sweat rashes, which may mimic mycotic diseases very closely. These pitfalls could easily be avoided by microscopical examination, which requires a 2/3 inch and 1/8 inch objective, a few drops of liq. potassae and a little experience and patience.

We will now pass to more difficult problem, that is the treatment of fungus infection of the hairy parts. Here the fungus, alighting on the surface of the skin, grows down the infundibulae of the hair follicles and then passes inwards to the adjacent hair shaft, insinuates itself between the cuticular cells

and either proliferates mainly around the hair, within it, or both within and without the hair. The first is characteristic of the *Microsporum* and *Trichophyton ectothrix*, the second of *Trichophyton endothrix* and *Achorion*, and the third the small *Trichophyton ecto-endothrix* group.

Let us deal first with infections due to the *Microsporum*. It was at one time the practice to treat all *Microsporum* infections in the same way but in recent years it has been realized that infection with animal microspora are more readily responsive to treatment than the commoner *Microsporum audouinii*, which appears to have acquired a very specialized parasiticism in man. It is essential, therefore, before embarking on treatment, to ascertain by cultural methods the variety of the fungus. If this proves to be of the human variety local measures will prove of little, if any, worth and unless the patient is approaching puberty, when the infection tends to clear up spontaneously, one will have to resort to more drastic methods. The commonest procedure, particularly in this country, is the irradiation of the whole scalp with X-ray with the object of causing a temporary hairfall. The correct dose, however, leaves only a very small margin of error and as faulty irradiation may give rise to permanent baldness it is prudent to have treatment conducted by those expert in this field.

Many years ago it was observed, whilst treating cases of phthisis with thallium, that there was occasionally a temporary hairfall. It was later determined that this hairfall took place with considerable regularity in 16 to 19 days after the administration of thallium acetate in a dosage of 8 mg per kg body weight. It was found later advisable to reduce this figure to 7.5 and even 7 mg in patients approaching puberty in which the drug, relatively toxic in any case, could produce poisonous effects. While this method has the advantage of simplicity, it should only be given with great care and never to patients with any evidence of renal disease. Its use in this country was discontinued about 20 years ago following the death of three children, but it should be mentioned, in justification of this method, that in that particular instance there was a gross error in the calculations which led to a very large overdose being given. Nevertheless, large epidemics of tinea tonsurans have been treated with thallium with success and the late GIOVANNI TRUFFI, of Padua, informed me that they had treated in that town several thousand cases without a fatality, the only untoward reaction being a transient arthritis. There is, however, another disadvantage of thallium over X-ray and that is that the hairfall frequently does not affect the anterior fringe of the hair margin and that, as hair regrowth is more rapid, unless it is encouraged to come away by additional physical means and the surface of the scalp well disinfected, re-infection of the newly growing hair may occur.

As one will gather, although these methods of X-ray and thallium can, in expert hands, produce excellent results, both have their hazards and if the infection is due to a fungus which can be eradicated by simpler methods they

should always be resorted to. This applies particularly to cat ringworm infection, the relative incidence of which appears to have increased in this country during the last two decades. *Microsporum felis* unlike *Microsporum audouinii* which grows on its host with practically no evidence of tissue reaction, produces a varying degree of inflammation which, if increased by external irritants, may cause local hairfall. When once the correct diagnosis has been confirmed the scalp should be clipped short, the infected areas determined and ringed with indelible pencil and rubbed vigorously with a mild chrysarobin ointment twice daily. As soon as loosening of the hairs is produced they should be epilated with suitable forceps or if the involved areas are large, with adhesive strapping. Owing, however to the high degree of infection of cat ringworm, repeated examination of the scalp under Wood's light should be made to determine whether new foci of infection have developed. Success with phenyl mercuric nitrate in a carbowax and crill base has been claimed by DRAIN, CROW, HABER and others, not only in animal infection of the scalp but even in six out of eight cases of infection with the human variety and I think it fully worth while trying this further as an alternative to the more irritating anthrarcobin compounds.

Infection with animal trichophyta of the ectothrix group almost invariably produces keria, which are local inflammatory reactions taking the form of raised, boggy swellings. Fortunately the inflammation usually causes a loosening of the hair with spontaneous cure, but if the reaction is inadequate it can be accelerated by the local application of tincture of iodine.

Infection with the *Trichophyton endothrix* group can prove very resistant to treatment and as the hairs so infected tend to break at the level of the skin, physical epilation is by no means easy even after the application of local irritants, and one must frequently resort to X ray therapy. The same applies to *Achorion* infection, which, despite treatment, frequently persists for many years, leaving permanent scarring in its trail.

Attempts to treat ringworm infection of the scalp by injection of an extract of fungi, referred to as trichophytin, to which patients with animal infection are usually allergic, have proved disappointing except in cases of keria due to animal trichophyta. The degree of allergy in these cases is frequently considerable and the intra-cutaneous injection of even small quantities, say 0.1 c.c. of 1 in 1000 solution of trichophytin, may not only elicit a strong reaction at the site of inoculation but also an inflammation of the kerion itself and thus help local hairfall.

We now come to the third group of fungus diseases, the onychomycoses or ringworm of the nails. Here we have soil which when once infected becomes an excellent medium on which the fungus can grow indefinitely without hindrance. In addition, the rate of growth of the fungus is invariably greater than that of the nail plate and therefore repeated paring of the nails does not keep pace with the rate of invasion. Further the mycelium grows, not only

made for the use of local measures, but if these cases were investigated they would probably be found to be examples of psoriasis of which may resemble onychomycosis very closely and which may solution with the normal growth of the nail. Once again verification of diagnosis by a microscopical examination should always be conducted. One who appreciates the dense nature of the nail, the futility of any local treatment will readily be realized. Unfortunately, fungus infection of the nail, particularly when it involves the fingers, is a very unsightly affection and treatment even though it may be of a radical nature. Apart from this, it is invariably a progressive affection, spreading slowly from nail to nail and may be responsible for recurring infections of the skin of the toes surrounding the infected nails. Temporary avulsion of the nail is resorted to in the first instance, but radical as this method appears notwithstanding thorough post-operative treatment, re-infection of the growing nail not infrequently follows. In by far the greater number of cases one has finally to recommend permanent avulsion of the nail. The removal of the nail plate with its matrix and, although it may be the case of the toe nails, one is naturally loath to order this for infection of the finger nails.

In dealing with the clinical aspect of this subject, mention should be made of allergic skin affections associated with fungus infections. It has been for many years that the affection previously recognized as cheilopompholytic and manifested by the development of a symmetrical vesicular eruption on the hands, may appear in patients with ringworm infection, particularly on the feet. Later, other skin lesions such as a scarlatiniform desquamation of the hands, were found to bear a similar relationship to dermatomycoses and, recently, some cases of erythema nodosum and rashes resembling lichen planus or pityriasis rosea, were proved to be allergic reactions to fungi. These cases merely denote a heightened sensitivity to the causal fungus and usually require no treatment, disappearing spontaneously after satisfactory treatment of the primary infection.

It must be very apparent that only the fringe of the field of therapy with fungus infections has yet been explored. Whether it may prove profitable to enquire along lines similar to those investigated in the case of bacterial infections is very doubtful owing to the horny site chosen for the infection of the fungus. WEIDMAN, before the introduction of penicillin, observed that *Trichophyton rubrum* was frequently found in toe interspaces not infected with fungus and on the assumption that the bacillus produced a substance inimical to the growth of fungi, sowed this organism between the infected toes, but his results were by no means convincing. Some years ago, working with WEBB,

MUENZER pointed out) and I feel everyone must agree that a survey of the fungus diseases in tropical Africa is long overdue.

Sir George McRobert Dr DUNCAN has confined his remarks to conditions prevailing in the British Colonial Empire. As one familiar with Burma and India, I should like to appeal for extensive and intensive work on medical mycology in warm climates generally.

Despite the existence of fine, well-equipped medical colleges and research institutes in Burma and India, with the fullest facilities for investigations in bacteriology protozoology and virus pathology it is almost impossible to get a specialist opinion on medical mycology in these countries—though the forestry and agricultural departments maintain their own mycologists.

Changes in the structure and nature of the Commonwealth make it more than ever essential for the London School of Tropical Medicine to maintain its position as fountain head of knowledge in tropical sciences. I should therefore welcome additional requirements in mycology for our diploma in tropical medicine.

Dr MUENZER has dealt chiefly with mycotic infections of the skin and hair but has done so from the point of view of the specialist working in a temperate climate. In the perennial hot and moist climates of deltaic Burma, Malaya, Ceylon and Southern India mycotic infections of the skin are almost universally present in the native population, though frequently in a mild and chronic form. In these areas the European who perspires freely readily becomes heavily infected on account of the difficulty in maintaining a dry skin; treatment presents a different problem from that met with in temperate climates. Removal to a cool hill climate is a great help in the treatment of such cases in the tropics and in really bad cases it is often sound policy to send the patient to Europe by air.

I agree with Dr MUENZER in his plea for early search for microscopic evidence of the presence of a fungus before treatment is instituted as I too, have seen many cases of dermatoses treated vigorously and unsuccessfully in the mistaken belief that a fungus was the cause of the trouble. Over treatment is very common. The majority of the cases who required urgent admission to the beds of my dermatological colleagues in Madras had been over-treated by their private practitioners and required mild permanganate baths and calamine lotion for some days.

I was interested to hear Dr MUENZER condemn active treatment of the hand lesions in cheilopompholyx associated with mycotic infections of the feet. My own experience is that lemon juice to the hands and fungicide to the feet is the best line to follow.

In the extensive mycotic infections of the glabrous skin of British troops in South India during the recent war the army dermatologists prescribed X-ray treatment in many resistant cases. Two 120 Kv treatment sets in my hospital in Madras were kept busy treating queues of infected soldiers. I was not

impressed by the results and should be interested to have Dr MUENDE's views

I should be interested, too, to hear his views on the value of the new anti-histamine drugs such as benadryl in the treatment of allergic manifestations in the skin

Diseases of the skin play a most important part in medical practice in the tropics and in a high proportion of these a fungus is the cause. In the major wealthy and populous cities there are, however, very few specialist dermatologists. In these cities there are fine openings and potentially lucrative fields for the practice of dermatology—a veritable dermatologist's paradise, with patients whose troubles respond to the ministrations of the specialist, but who always come back again sooner or later!

Dr H S Stannus pointed out the great frequency of fungus diseases in Central Africa. A survey, he considered, would be extremely interesting, but the discovery of effective treatment is much more important.

Dr J T Duncan (in reply). Firstly, I would like to thank Dr MUENDE for his kindly reference to my work on parasitic diseases of the skin, but I must point out that the major credit for this work should be accorded to my assistant, Mrs WALKER. Dr MUENDE compares the brilliant results of chemotherapy in bacterial infections with the less scientific approach to the problem of curing fungal diseases. There is, however, nothing amongst the mycoses comparable to the acute bacterial infections in which the most spectacular chemotherapeutic successes have been obtained. In fact, the systemic mycoses tend to conform to a common pathological pattern which links them to such bacterial diseases as tuberculosis and leprosy, and, on the whole, I think it may be said that the chemotherapy of the systemic mycoses has not been less successful than that of tuberculosis or leprosy. The value of natural antibiotics in the treatment of fungal diseases has also been questioned, but penicillin, in large doses, is known to be the best treatment for some forms of actinomycosis, although its use in moniliasis may have harmful results. Dr STANNUS has mentioned the frequency of apparent fungus diseases of the skin in tropical Africa, and he considers that a survey of these diseases would be helpful. Other speakers in the discussion have also considered fungus infections only in relation to the skin, but, in advocating a survey of fungus diseases in the colonies, I was thinking chiefly of the systemic mycoses which are likely to be entirely overlooked or mistaken for tuberculosis and other granulomatous diseases, unless a proper mycological examination is made. Cases of suspected tuberculosis in which the diagnosis is not confirmed by the results of bacteriological examinations should be examined mycologically. As an example, I would quote the case of some men serving in the Near East who were invalided home on the clinical diagnosis of tuberculosis supported by X-ray photographs showing cavitation of the lung. When these men were examined on their return to England no sign of tuberculosis could be found, the lung cavities had

disappeared and as the patients were apparently in good health they were discharged fit for duty and there the matter ended. The clinical history of these cases should have raised the suspicion of primary pulmonary coccidioidomycosis, which could have been settled by test of dermal sensitivity to coccidioidin. The omission to perform this simple mycological test is to be regretted as it might have yielded valuable evidence bearing on the question of the supposed focus of the coccidioid disease in the Mediterranean area.

Dr. Munde (in reply) It is certainly very regrettable that there is no uniformity in nomenclature, but this is due to the fact that dermatologists and physicians generally have tended to retain the names first suggested by SABOURAUD.

X ray therapy is of no value in fungus infection of the glabrous skin. Even in large doses it can only alter the mode of growth of the fungus and if the dose was sufficiently large to kill the organism severe destructive changes would occur in the epidermis too.

With regard to cherropompholyx, I would like to stress that this merely denotes a symmetrical vesicular eruption of the hands and that only a small proportion of such cases can be attributed to a state of allergy developing as a result of a mycotic infection elsewhere. If the diagnosis is correct, that is, that the cherropompholyx is essentially a vesicular mycoid, the latter should clear up spontaneously once the primary infection has been eradicated.

As to benadryl, I have not found it anything like as efficacious as others claim it to be.

Trichomyces axillaris is an affection which does not invade either the stratum corneum or the hair but merely encompasses the hair with a concretion which CASTELLANI said was due to the presence of a *Cohnistrepotrix* living in association with a coloured coccus and depending on the pigment of the coccus, so we have the three varieties of trichomyces axillaris, yellow red and black. Treatment should, therefore, be directed to shaving the hair. Applications applied to the skin are redundant and may if they are irritants, give rise to a dermatitis medicamentosa.

Most cases of ringworm infection are curable but recurrences may occur depending upon whether or not a patient exposes himself to re-infection.

I agree that tinea pedis is commoner among clean people and this can be attributed to the fact that they are more liable to contract infection from bath mats, etc. If we had a hypothetical case of a patient who never had a bath I think we would find that it would be very unlikely for him to contract tinea pedis.

COMMUNICATIONS.

THE LIFE CYCLE OF *PLASMODIUM CYNOMOLGI* IN ITS INSECT AND MAMMALIAN HOSTS

BY

H E SHORTT, M D, CH B, D SC, D T M & H, I M S (ret'd)

The recent discoveries made in connection with the exo-erythrocytic stages of mammalian malaria parasites of the genus *Plasmodium* (SHORTT and GARNHAM, 1948d, SHORTT, GARNHAM and MALAMOS, 1948a, SHORTT, GARNHAM, COVELL and SHUTE, 1948b, SHORTT and GARNHAM, 1948c, and SHORTT and GARNHAM, 1948d) have rendered necessary some fundamental additions to accounts describing the complete life cycle of members of this genus. As it is, the recent additions to our knowledge are approximately complete only in the case of *P. cynomolgi*, but there is no reason to suppose that the life-cycles of other members of the genus whose morphology and general behaviour in the mammalian host are essentially similar will differ materially from that of *P. cynomolgi*. It is for this reason and to place the newer knowledge in its proper relation to previously known details of the life cycle, that the present description is given to serve as a type for comparison with the life cycles of other species of the genus *Plasmodium* as these are elucidated.

In the account to be given below, no attempt is made to repeat descriptions of details in the life cycle which are already well known and only the recent additions to our knowledge will be incorporated in their proper sequence in the complete life-history of *P. cynomolgi*.

LIFE HISTORY OF *Plasmodium cynomolgi*

This is divided into two parts, that passed in the insect host and that passed in the vertebrate host, but a more comprehensive view of the whole process will be obtained if we consider the complete sequence of events as a continuous cycle embracing the various stages passed through in these two situations.

The term life-cycle indicates that a start can be made at any point and that if the sequence of events is followed continuously, a return to that point will be reached.

A convenient place at which to begin is the act of biting by the normal anopheline mosquito when it sucks blood from a monkey infected with *P. cynomolgi* and carrying the male and female gametocytes of this parasite in its blood. After the blood has entered the mid-gut of the mosquito, the asexual

and the immature sexual forms of the parasite are destroyed by the digestive processes.

The mature sexual forms, the gametocytes, undergo the well-known stages of development, commencing with reduction division to produce gametes, followed by fertilisation of the female gamete by the male, to form the zygote, and proceeding through the stages of ookinete oöcyst (now on the outer surface of the mid-gut), formation of sporozoites in the oöcyst and invasion of the salivary glands of the mosquito by these sporozoites which are the form infective to the vertebrate host. It is at this stage that recent findings have to be interpolated in the older picture of the life history and they refer to the development of the sporozoites inoculated into the vertebrate host when the mosquito bites to suck blood.

The sporozoites are inoculated into the tissues and may be found circulating in the peripheral blood for about half an hour but thereafter disappear and are no longer demonstrable by inoculation of even large quantities of blood into a clean and susceptible host, as demonstrated by FAIRLEY (1945) for *P. vivax* in the Cairns experiments.

The reason for this disappearance is that the sporozoites have found a protected location in the parenchyma cells of the liver. The description to follow refers to the appearances presented in microscopical sections of liver tissue stained by Giemsa stain.

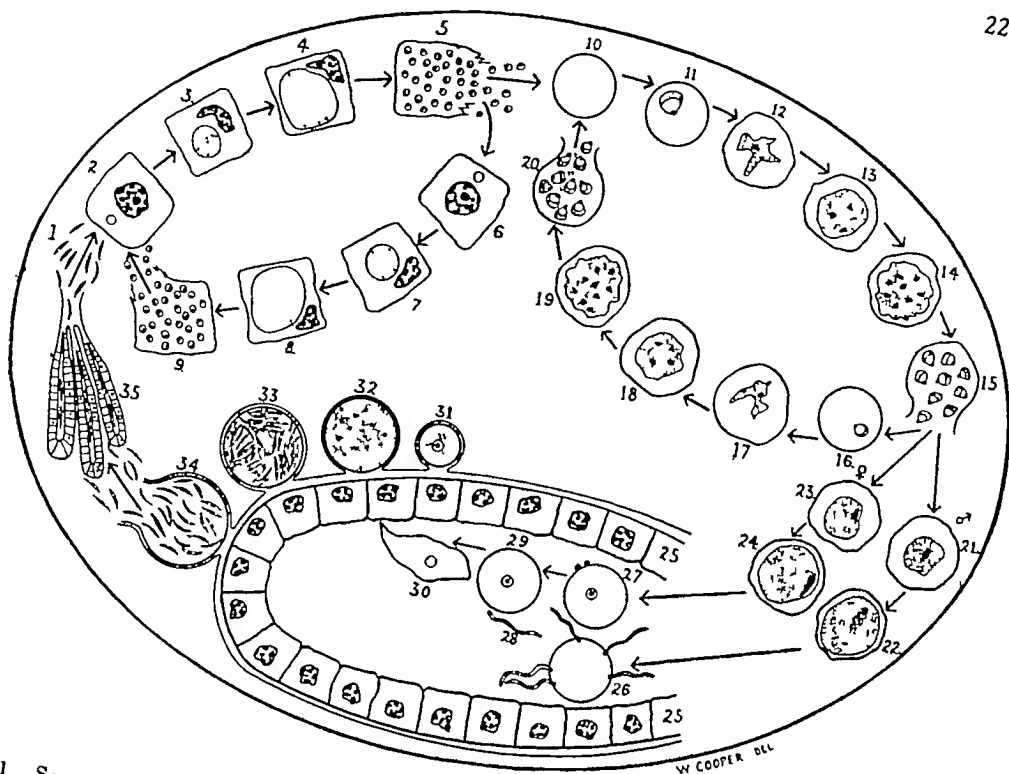
The earliest stage at which the parasites have been definitely observed in this situation is that reached by the fourth day after the bite of the mosquito. In sections of liver tissue these forms appear as more or less spherical bodies, about 10μ in diameter situated within the cytoplasm of parenchyma cells. The parasites have now assumed the status of schizonts and present 20 or more fragments of magenta-staining chromatin embedded in a pastel blue staining cytoplasm. These nuclei appear to be in process of active division.

After a further 24 hours, the parasite has increased in size to about 10.5μ in diameter with a corresponding increase in the size of the containing liver cell. The particles of chromatin have increased in number and the subsequent development takes the form of a rapid division of the chromatin particles until the latter may number over a thousand by the seventh or eighth day while the diameter of the parasite may now be as much as 40μ .

From the sixth day onwards in this development, many of the parasites contain one or more vacuoles while the spherical shape may give place to an ovoid or even less regular shape owing to unequal resistance at its periphery by surrounding structures. The increase in size of the parasites distends the containing liver cell until it is a mere membrane surrounding the former and the nucleus is pushed to the periphery.

The presence of the parasite and its growth in the liver tissue is unaccompanied by any reaction in the liver beyond the pressure exerted on the surrounding cells.

By the eighth day the cytoplasm in some of the schizonts has concentrated around the fragments of chromatin to form meronts which measure about



EXPLANATION OF PLATE

- 1 Sporozoites from salivary glands of mosquito enter liver cells
- 2 Liver cell containing early stage of pre-erythrocytic parasite
- 3 and 4 Stages in development of the pre-erythrocytic schizont in liver cells
- 5 Fully developed pre-erythrocytic schizont rupturing and releasing pre-erythrocytic merozoites
- 6 Liver cell containing merozoite of exo-erythrocytic cycle of schizogony
- 7-9 Remaining stages in exo-erythrocytic schizogony ending in second generation of merozoites
- 10 Red cell of circulating blood
- 11-14 Stages in erythrocytic schizogony in circulating blood
- 15 Fully developed erythrocytic schizont rupturing and releasing erythrocytic merozoites and gametocytes
- 16-20 Repetition of erythrocytic schizogony
- 21 and 22 Development of male gametocyte or microgametocyte in circulating blood
- 23 and 24 Development of female gametocyte or macrogametocyte in circulating blood
- 25 Wall of stomach of mosquito
- 26 Exflagellating microgametocyte producing microgametes in stomach of mosquito
- 27 Macrogametocyte extruding polar bodies and so becoming macrogamete
- 28 Microgamete free in stomach of mosquito and seeking macrogamete
- 29 Zygote, formed by fertilization of macrogamete by a single microgamete
- 30 Ookinete or travelling vermicle formed by elongation of zygote It is about to penetrate epithelial lining of stomach
- 31 Oöcyst, formed by ookinete after penetration of stomach wall of mosquito It lies under elastic membrane on outer surface of stomach
- 32 and 33 Stages in development of oöcyst with production of sporozoites
- 34 Rupture of mature oöcyst with dispersion of sporozoites most of which enter salivary glands of mosquito
- 35 Salivary gland of mosquito containing mature sporozoites

1 13μ in diameter. The rupture of the schizont releases these into the surrounding tissues. Some of the merozoites now enter red cells of the blood to initiate the blood infection. These undergo the well known stages of schizogony in the erythrocytes of the circulating blood with the production of asexual and sexual parasites accompanied by the clinical effects of fever and anaemia in the host.

Other merozoites maintain the exo-erythrocytic cycle by entering fresh liver cells where they undergo a cycle of schizogony similar to that described for the primary schizonts. The process of maturation of the schizonts in the liver is not completely synchronous and it may be said, as a general statement, that merozoites are being liberated from the eighth to eleventh days after the original infection with sporozoites.

The immunity reactions of the host to these two simultaneously occurring cycles of development, erythrocytic and exo-erythrocytic, are not yet fully understood but appear to be exerted mainly on the erythrocytic parasites. Thus, after a varying number of exacerbations of the infection, the active stage of the disease dies down and parasites disappear from the peripheral blood or become extremely scanty as the result of the immune response of the host. The exo-erythrocytic cycle, however on account of the relative inaccessibility of the intracellular parasites, would appear to be more protected against the immunity mechanism and able to maintain itself even when the erythrocytic cycle has been suppressed whether by the host's acquired immunity or by anti-malarial drugs. Those merozoites produced by it which are destined to maintain the exo-erythrocytic cycle, immediately gain the protection of liver cells while those destined to enter red cells are overwhelmed by the specific immunity of the host against the erythrocytic parasites.

The mechanism would appear to offer a reasonable explanation of the occurrence of relapses. When, for any reason, the host's specific immunity becomes lowered the merozoites from the liver destined to enter erythrocytes are no longer destroyed and an erythrocytic relapse is initiated.

The foregoing very brief description now enables one to represent diagrammatically the full story of a malarial infection produced in a monkey by *P. cynomolgi* and this is attempted in the diagram accompanying the paper.

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A PRELIMINARY REPORT ON THE VALUE OF PALM-LEAF TRAPS IN THE SURVEY AND TREATMENT OF STREAMS INFESTED WITH SNAILS

BY

M ABDEL AZIM, D V M S , D T M C H
AND

NAGUIB AYYAD, M D , B M S

Bilharzia Snail Destruction Section, Egyptian Ministry of Public Health

It is well known that aquatic snails attach themselves to floating and partially decaying vegetable matter such as cornstalks, sugar cane, sticks and branches, banana or palm leaves, where they feed and deposit eggs. This fact suggested the possible use of such matter as snail traps. The most suitable for the purpose in this country are palm leaves, which are obtainable everywhere and at all seasons very cheaply or free of charge. They are also well liked by the snails, present a large surface and can be used for several weeks before disintegrating.

The survey of streams for *Bulinus truncatus* snails by traps was first applied by the Bilharzia Snail Destruction Section in large main canals with a snail population so scanty that dipping by net gave negative results. In the case of smaller streams with heavy infestation dipping is still the regular method for obtaining quick results.

Later, the traps were used in some streams of the Fayoum for surveys, to show seasonal fluctuations in the snail population as well as the effect of control measures. This method of survey, being successful in catching snails, suggested the regular use of traps for snail removal. For this purpose traps were first tried in Bahr Youssef, the main feeding canal of Fayoum Province, during the winter of 1943-44. Subsequently they were tried in several other main streams and proved so satisfactory that they are now recognized as a regular method for snail removal.

The use of traps for treatment is especially convenient whenever treatment of streams by our usual methods, i.e., clearance and sulphation is not possible owing to difficulties of water control. Streams cannot be cleared or dammed for sulphation unless the water is shut off. At certain seasons and in certain districts it is impossible to obtain closure of the intakes of canals, and this

applies above all to the Fayoum, a rice and fruit growing centre. The water conditions in this province are most unfavourable and further complicated by the absence of an intermediate dry period between irrigation rotations, and the difference of level between "high" and "low" water is also insignificant.

1. EXPERIMENTS.

In the following, series of experiments are reported throwing light on questions such as

- (1) To what extent are snails carried by the large streams and at what levels?
- (2) What proportion of snails will be caught by traps in the presence or absence of other food stuff?
- (3) What is the distribution of snails in stream under various conditions of current, depth, etc.?

1. EXPERIMENTS IN BAHR YOUSSEF

Two experiments were carried out in Bahr Yousef to estimate the number of snails being carried into Fayoum Province by its main feeding canal during the flood and in other seasons at different water levels of the stream. The site of the experiment was bridge in Fayoum town, opposite the Police Station and near the Post Office where the stream is about 22 m. wide and 2 m. deep. (Low rotation 185 cm high rotation, 195 cm. flood, 220 cm.)

(a) The experiment was carried out during the flood, from 25.8.45, to 13.9.45. A set of palm leaves closely chained together in parallel and forming dense curtain was suspended by four ropes from the left side of the bridge. This set was composed of 13 rows of leaves and intersected only portion of the upper and middle layers of the stream, well clearing the bottom. Another set was lowered into the canal near the first, touching the very bottom and screening the deeper layers only (Fig. 1).

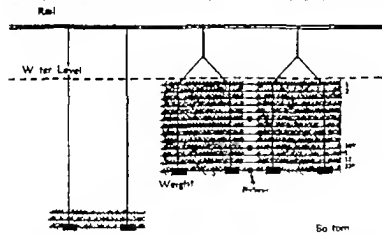


FIG. 1
Cross Section
of
Bahr Yousef.

The traps were inspected after 8 days (Table I), and were then removed and suspended on the right side of the bridge. An inspection count was made 11 days later given in the table. The lowest palm leaf in the set had then broken off and was trailing on the bottom of the stream.

It is seen that *Balorus* were found in the middle layers (6th to 13th leaf) but the current was too strong for great number of snails to remain attached to the leaves in the experiment was carried out during the height of the Nile flood.

TABLE I
SHOWING THE NUMBER OF SNAILS CAUGHT IN BAHR YOUSSEF

Ladder	Leaf	Left side							
		Inspection count after 8 days							
		<i>Bulinus truncatus</i>	<i>Melania tuberculata</i>	<i>Lanistes bolteni</i>	<i>Neritina nilotica</i>	<i>Cleopatra bulimoides</i>	<i>Vivipara umcolor</i>	<i>Pyrgophysa forskahli</i>	<i>Limnaea caulliaudi</i>
Upper	1	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0
	5	0	0	0	0	0	0	0	0
	6	0	0	0	0	0	0	0	0
	7	0	0	0	0	0	0	0	0
	8	0	0	0	0	0	0	0	0
	9	0	0	0	0	0	0	0	0
	10	2	0	0	0	0	0	0	0
	11	0	0	0	0	0	0	0	0
	12	0	0	0	0	0	0	0	0
	13	3	4	0	0	0	0	0	0
		5	4	0	0	0	0	0	0
Bottom		0	1	1	59	0	0	0	0
Total		5	5	1	59	0	0	0	0

Ladder	Leaf	Right side							
		Inspection count after 11 days							
		<i>B. truncatus</i>	<i>M. tuberculata</i>	<i>L. bolteni</i>	<i>N. nilotica</i>	<i>C. bulimoides</i>	<i>V. umcolor</i>	<i>P. forskahli</i>	<i>L. caulliaudi</i>
Upper	1	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0
	5	0	0	0	0	0	0	0	0
	6	2	1	0	0	1	0	0	0
	7	0	0	0	0	0	0	0	2
	8	0	0	0	0	3	0	1	0
	9	0	0	0	0	0	0	0	0
	10	0	0	0	0	0	0	0	0
	11	0	0	0	0	0	0	0	0
	12	0	0	0	0	0	0	0	0
	13	2	2	0	0	0	0	0	0
		4	3	0	0	4	1	0	0
Bottom		0	0	0	0	4	1	1	2
Total		4	3	0	0	0	0	0	0

(b) In the last days of October after the flood two palm-leaf ladders were suspended from the same bridge one on the right, the other on the left side. Each ladder had ten rungs, about 50 cm. apart, and each rung was formed of two superimposed palm leaves approximately 2 m. long and pointing in opposite directions (Fig. 2a). The ladders were loaded with lead weights and touched the bottom of the stream, clearing the slopes, but they were deflected in great bulge by the force of the current (Fig. 2b).

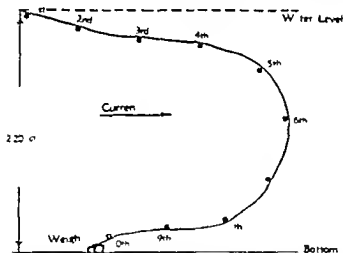
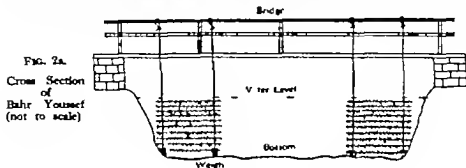


FIG. 2b

Profile showing deflection of the ladder by current and depth of the rungs.

Table II shows the number of snails, *B. truncatus* and other species, caught on each rung of the two ladders in nine inspection counts. It is seen that the number of *Bulimus* as well as the total number of snails from all species, diminished from November the date of the first count, to the end of December when the experiment was interrupted owing to the winter closure while the snails increased from April onwards when the experiment was resumed. Most *Bulimus* were caught near the surface none below the eighth rung.

II. EXPERIMENT IN UPER HUNG WATER (10 H 43 to 22.8.46)

Two ladders were suspended from the rail of bridge about 1 km from the intake of the canal from Bahr Yousef where it is about 22 m. wide and 2.5 m. deep. Each was composed of ten rungs, 60 cm. apart, and weighted to touch the bottom. The excess length bulged downstream, just as in the preceding experiment.

TABLE II
SHOWING THE TOTAL NUMBER OF SNAILS CAUGHT ON BOTH LADDERS IN BAHR YOUSSEF

Number of rung	1945					1946				Totals
	5 11	24 11	1 12	16 12	23 12	13 4	8 6	10 6	9 7	
	<i>B. truncatus</i> Other snails	<i>B. truncatus</i> Other snails	<i>B. truncatus</i> Other snails	<i>B. truncatus</i> Other snails	<i>B. truncatus</i> Other snails	<i>B. truncatus</i> Other snails	<i>B. truncatus</i> Other snails	<i>B. truncatus</i> Other snails	<i>B. truncatus</i> Other snails	
1	4 3	3 9	0 1	0 0	0 0	11 15	1 0	4 4	6 16	20 48
2	0 5	3 14	3 4	0 1	0 0	0 2	1 0	7 6	1 1	15 33
3	0 1	0 0	0 2	1 0	0 0	0 0	0 0	1 2	0 4	2 0
4	0 0	0 0	0 2	0 0	0 0	0 0	0 0	1 1	3 4	4 7
5	0 2	0 3	1 0	0 2	0 1	0 2	0 0	0 0	2 0	3 10
6	0 1	0 0	0 0	0 0	0 0	0 0	0 0	0 1	5 3	5 5
7	0 2	0 0	0 0	0 1	0 1	0 0	0 0	3 3	2 3	5 10
8	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	2 1
9	0 4	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 4
10	0 15	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 15
Totals	4 33	6 26	4 9	0 6	0 2	13 19	2 0	16 17	19 31	65 142

The experiment lasted from October, 1945, until June, 1946. The eight inspection counts which were made showed that very few snails had been caught. The total of seven *Bulinus* were all caught on the topmost rung and in spring.

III EXPERIMENT IN TERET EL GIZAWIYA (10 10 45 to 16 6 46)

The experiment is similar to the two preceding ones. Two ladders were suspended from a bridge 2 km from the intake of the canal, where it is 25 m wide and 3.5 m deep (low rotation 3.2 m), all other conditions being alike.

The seven inspection counts made in the whole period from October to June showed that only two *Bulinus* were caught—both in April—on the first and fourth rung of the traps, that is within 80 cm of the surface.

IV EXPERIMENT IN BAHR EL GHARBIYA (9 8 45 to 18 8 45)

A ladder composed of 11 palm leaves was attached to a bridge crossing the canal 4 km from the intake where its width is 8 m and the depth about 140 cm. The current

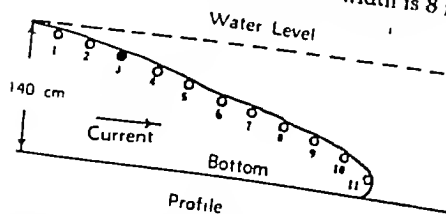


FIG 3
Ladder in Bahr
el Gharbiya,
profile and
plan

at the time was so strong that the upper portion of the ladder assumed an inclination of about 20° to the horizontal. Each leaf of the ladder was bent by the force of the current, striking it perpendicularly (Fig 3).

The ladder was examined for snails 10 days later with the following results

TABLE III.

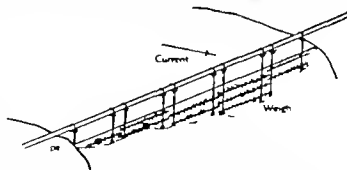
Leaf.	<i>B. truncatus</i> .	<i>P. forshskali</i> .	<i>A. natica</i> .	<i>Physa acuta</i> .	<i>Planorbis marginatus</i> .
1	0	0	0	0	0
2	0	0	0	0	0
3	1	1	2	1	0
4	0	0	12	0	0
5	0	0	12	1	1
6	0	0	2	0	0
7	0	0	0	0	0
8	0	0	0	0	0
9	0	0	0	0	0
10	0	0	2	0	0
11	0	0	0	0	0
Totals	1	1	27		1

It is seen that no snails were found on the very surface but that some snails were carried by the current in the upper layers (third to fifth rung). Only one *Balanus* was caught. It occurs that except for *Neritina*, which are usually found in the lower levels, the snails were not able to remain attached in so strong a current.

V. EXPERIMENT IN AL-BEIR EL-A'LAM (12.6.45 TO 19.6.45)

The site of the experiment was sharp bend in Beir el A'lam, 5 km. from the intake where the canal is 13 m. wide and reaches depth of 1.5 m. on the outer side of the bend.

FIG. 4
Cross section of Beir
el A'lam bend.



the side where the current is strongest. From a pipe that crosses the canal at this point, five palm-leaf ladders were suspended side by side to screen the cross-section of the canal. These ladders had one, two or three rungs, according to the depth of the bottom (Fig. 4)

A count was made after 7 days with the result shown

TABLE IV

Ladder	Leaves	<i>B truncatus</i>	<i>C bulmoides</i>	<i>M tuberculata</i>
1	1	2	19	1
2	1 2	0 1	0 3	0 1
3	1 2	0 0	0 12	0 6
4	1 2 3	0 0 0	0 0 0	0 0 0
5	1 2	0 0	0 0	0 0
Totals		3	34	8

Three *Bulmus* were caught. These, as well as the snails of the other species, were present only at the bottom of the inside bank of the bend where the water is shallow and the current moderate.

TABLE V

Leaf	1st inspection after 8 days					2nd inspection after 15 days				
	<i>B truncatus</i>	<i>C bulmoides</i>	<i>L bol-tem</i>	<i>P mareo-ticus</i>	<i>N mlo-tica</i>	<i>B truncatus</i>	<i>C bulmoides</i>	<i>L bol-tem</i>	<i>P mareo-ticus</i>	<i>N mlo-tica</i>
1	1	2	3	0	0	0	0	0	0	0
2	0	0	0	0	1	0	0	0	0	0
3	0	0	1	0	1	5	0	0	0	34
4	3	0	1	0	1	9	0	0	5	150
Totals	4	2	5	0	3	14	0	0	5	184

VI EXPERIMENT IN DAHR EL BASHAWAT (28.8.45 to 4.9.45)

A ladder of four palm leaves was attached to a bridge crossing Bahr el Bashawat, a medium-sized stream in the Fayoum, whose depth in flood time reaches 115 cm (Fig 5). The bridge is situated 8 km from the intake and the canal is 5 m wide. The current was so strong that it was difficult to fasten the trap and maintain it.

The trap was inspected after 8 days and a second time 15 days later with the following result

Most of the snails caught were found in the lower half of the canal, whose depth does not reach that of the larger main stream. Eighteen *Balanus* were recovered and large number of *Vermetus* which do not seem disturbed by strong currents

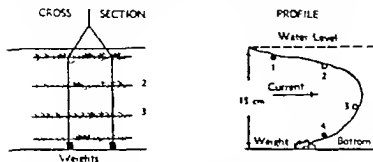


FIG. 5

Behr & Bhattacharya

VII. EXPERIMENT IN EL AGOZA CANAL (11.8.45 to 18.6.46)

The experiment was carried in el Agouza canal, shallow weedy stream in the F yomen, to compare the number of snails caught by traps in a part of the canal which had been weeded, with the number of snails caught in a weedy portion. During the course of the experiment a certain portion of the canal, about 8 km. from the intake was cleared in three intervals of about 2 to 3 months, coinciding with every other inspection count. The clearance was superficial in so far as only the weeds along the banks were removed, without disturbing the canal too much. The width of the canal the site of the experiment is about 7.5 m. and its depth during flood about 80 cm. normally it fluctuates between 50 and 60 cm.

A set of four strings was laid across the canal at the centre of the cleared portion ("A" in Fig. 6) each string being composed of three leaves tied end to end and weighted. The distance between strings was 1.5 m. Another set of four strings was placed in the weedy portion ("B" in Fig. 6). The current was flowing from the cleared to the weedy portion.

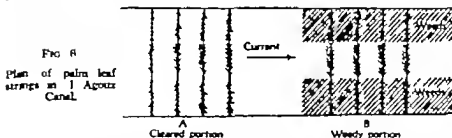


FIG. 6

Plan of palm leaf strings in El Agouza Canal.

From Table VI it is seen that the traps function very satisfactorily even in the presence of natural food supply. Approximately equal numbers of *Balanus* were caught in the cleared and weedy portion. Considering the total catch of all species the cleared portion yielded about twice as many snails in the weedy portion. In all cases the snails were evenly distributed over the slopes and bottom of the canal. The number of snails caught decreased steadily until the end of the year but rose again from the following June.

TABLE VI
SHOWING THE NUMBER OF SNAILS CAUGHT IN AGOUZ CANAL

Date of collection	A. Cleared portion			B Weedy portion		
	<i>Bulinus</i>	Other snails	Totals	<i>Bulinus</i>	Other snails	Totals
8 8 45	52	10,024	10,076	130	2,422	2,552
28 8 45	19	2,052	2,071	71	1,545	1,616
11 10 45	31	1,339	1,370	30	1,011	1,041
15 12 45	54	1,757	1,811	53	858	911
23 12 45	37	689	726	48	697	745
Winter closure						
10 5 46	0	273	273	0	69	69
8 6 46	91	430	541	60	314	374
16 6 46	128	588	716	128	454	582
11 7 46	179	664	843	158	511	669
29 7 46	231	378	609	199	262	461
10 8 46	120	1,000	1,120	42	561	603
Totals	942	19 214	20 156	919	8 707	9 626

VIII EXPERIMENT IN BAHR EL ZAWIYA (23 7 45 to 2 8 45)

Four hundred metres from the intake of Bahr el Zawiya, two strings of palm leaves, 50 m apart, were laid across the canal, following the contours of bank and bottom of the canal bed. The strings were composed one of six, the other of four palm leaves, and the width of the canal at the position of the strings was 11.5 and 8.6 m respectively, the depth not quite 2 m in both cases (Fig. 7).

After 6 days the snails attached to the leaves were counted, with the following results

TABLE VII

String	Leaf	Depth from surface in metres	<i>B. truncatus</i>	<i>C. bulbimoides</i>	<i>M. tuberculata</i>	<i>Balvata nilotica</i>	<i>L. boltemi</i>	<i>N. nilotica</i>	<i>P. mareoticus</i>
1	1	0	0	18	0	0	0	0	0
	2	0	0	21	0	1	0	0	0
	3	0.8	7	3	1	4	2	1	0
	4	1.8	5	38	0	0	0	0	0
	5	1	1	8	2	1	0	0	1
	6	0	0	4	0	0	0	0	0
2	1	0	0	0	2	0	0	0	0
	2	0.5	1	19	0	0	0	0	0
	3	1.2	4	20	0	0	0	0	0
	4	0.8	2	15	0	0	0	0	0
			20	146	5	6	2	1	1

It is seen that no *Bulinus* were caught on the shallow borders near the surface but that all were caught on the bottom, at depths varying between 50 and 180 cm.

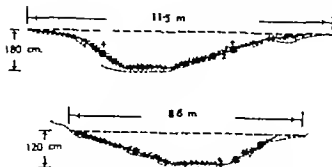


FIG. 7
Cross section of Bahr
el Zakhir at location of
strings.

IX. EXPERIMENT IN BAHR SENNOURES (23.8.45 to 6.9.45).

This main canal was cleared, during the flood, for a stretch of 80 m. on either side of bridge situated about 3 km. from the intake of the canal. The cleared part was surveyed by net but showed no snails.

A ladder was made by tying 21 palm leaves to two ropes. The leaves averaged 3 m. in length and were tied 0.5 m. apart. The ladder was placed across the canal, following the contours of its bed in a part where the canal was 15.2 m. wide and 2.2 m. deep (Fig. 8a and b).

Thirteen days later the ladder was taken out and examined and the number of *Bulinus* caught was counted (Fig. 8a).

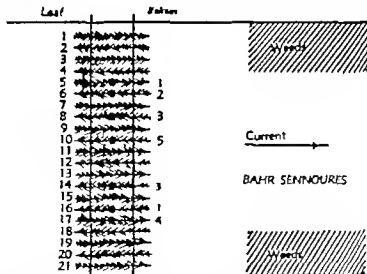
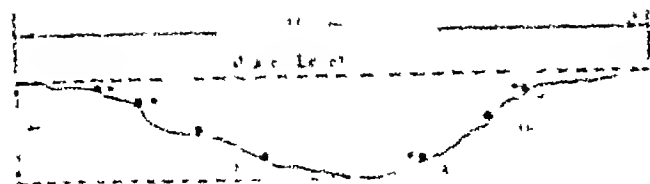


FIG. 8a.
Plan of ladder
laid across Bahr
Sennoures.

It is seen from the experiment that:

(1) Large streams which are clean from weeds and negative for snails by dipping, may show snails when traps are left for certain time.

(2) The main catch of snails is on the slopes, and not in the deep central channel the shallow parts near the banks.



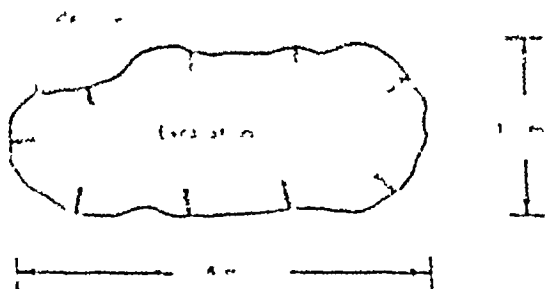
The results of the
S. G. after showing the
proof of the failure
on which H. G. were
causing

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7-1189

Year	Number of Deaths	Number of Cases	Ratio
2004	1	0	143.0 (crossed out) and (0.0 = 0.0)
2005	4		
2006	1		

1 2 3 4



AN EXPERIMENT IN A LABORATORY FOR THE

The experiment was carried out in one of the laboratory ponds resembling a tertiary canal, fed with Nile water and having a water capacity of about 12 cubic metres. The water was running part of the time. 2,500 *B. truncatus* and 500 *P. leuiscus* were placed in the pond together with 16 palm leaves each from 1 to 1.5 m long and about 1 m apart. The palm leaves were examined at intervals of 6 and 2 days to compare the length of time required for a good catch. The snails caught and the eggs or eggs deposited on the leaves were counted and removed and the leaves replaced into the pond. In about 1 month more than half of the *Budurus* and half of the *Planorbis* were caught. The number of snails caught decreased with each collection with few exceptions. At the end of the experiment only very few live snails were left and empty shells were found in numbers. Seven collection counts were made and the details of each are given in the following table.

TABLE IX.

Number of counts.	Number of days between counts.	Number of snails.		Number of egg-clusters.
		<i>Bulimus</i>	<i>Pisumia</i>	
1st	0	672	55	774
2nd	2	394	55	314
3rd	6	234	20	345
4th	2	69	6	116
5th	4	18	61	178
6th	2	26	6	61
7th	12	34	5	63
	25	1,490	241	1,791

CONCLUSIONS.

From the foregoing experiments it is safe to conclude that —

1. A small number of *Bulimus* are being carried in the middle and upper layers of waters of the main canals.

2. A fair proportion of *Bulimus* present in a stream can be caught with palm leaves, even in the presence of other aquatic vegetation as the palm leaves are very attractive to the snails, especially after the hard outside of the leaf has begun to soften through decay.

3. *Bulimus* are distributed equally over bottom and slopes of the canal bed in shallow canals (depth to 1 m.) especially when the canal is wide and the strength of the current broken by vegetation. They are more or less confined to the slopes in deeper and swifter canals. They are not able to withstand current to the same degree as, for instance, *Neritina nilotica*.

2. TREATMENT BY PALM LEAF TRAPS.

It has been mentioned already that snail removal by palm-leaf traps has been put into practice as a control measure in all cases where the control of water necessary for our usual methods of clearance and sulphation, is unpracticable.

Method of Treatment

1. The weeds are removed from both sides of the stream where the water is comparatively shallow with sickle and hoe, by our regular clearance gangs. As in our usual weed clearances, definite lengths are assigned according to the weed condition, and the weeds are thrown far up the banks to prevent any backsliding that would cause reinfection down-stream or provoke

obstructions at the intake of subsidiary streams. Before beginning clearance it is an advantage to remove snails by net.

2. Portions of palm leaves are then stuck into either bank below the surface of the lowest water level, about 1 m apart.

3. Three km of bank are assigned to one man, living in the neighbourhood, who is responsible for maintaining the traps, removing the snails and keeping his stretch permanently free from weeds. The collection of snails takes place at weekly intervals. The collectors are instructed to remove traps carefully so as not to drop snails into the water. The large snails are picked and counted, egg-clutches are removed. Then the traps are shaken vigorously on the bank to shake off such snails as have not been picked, and the traps are replaced.

Advantages

The main features recommending this mode of treatment are the following:

1. The elimination of the necessity for the closure of intakes as well as the applicability to all main canals, regardless of agricultural season.

2. Cheapness. The palm leaves can be obtained easily and free of charge in most cases. They can be used for about 2 months each and involve little labour. The periodical clearance or sulphation of big canals entails heavy expense with regard to labour, transport, equipment and chemicals.

3. Readiness for sulphation at any time. All main or secondary streams in which palm leaves are used are kept permanently clean from weeds. No time need be lost for weeding—necessary before sulphation—and sulphated waters can act for the whole period of low rotation giving best results.

4. Continuity of snail removal and survey. The method can be applied all the year round, the 40 days of "winter closure" of water excepted. At the same time the traps act as a means of continuous survey, and as they are but 1 m apart, they give a clear and accurate picture of environmental and seasonal fluctuations of the snail population.

Results Obtained in Main Streams

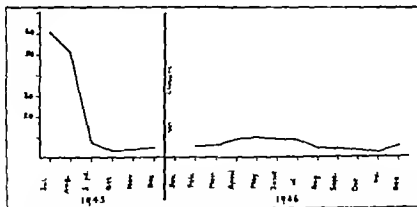
Between July, 1945, and December, 1946, 39 main streams in Fayoum Province, totalling approximately 450 km, were treated by means of palm-leaf traps and about 250,000 *Bulinus* were removed. Only six of these streams were treated by traps alone for the whole, or the greater part of this period. The rest were sulphated once or twice and not treated with traps for a certain time after the sulphation, or else snail collections were suspended for longer or shorter periods due to scantiness of snails, clearances, etc., 11 of these 39 streams were treated with palm-leaf traps in the last 6 months only.

An excellent example of continuous treatment by palm leaves can be found in Bahr El Bashawat, a heavily infested stream, 22 km in length, which was cleared only once in December, 1945 (Table X).

TABLE X.
NUMBER OF *Bulinus* COLLECTED MONTHLY FROM 8 KM EL BASHAN T BY MEANS
OF PALM-LEAF TRAPS.

Month.	Number of <i>Bulinus</i> .	Month.	Number of <i>Bulinus</i> .
1945 July	29,703	1946 May	1,643
August	59,234	June	1,200
September	1,820	July	692
October	413	August	214
November	177	September	64
December	Clearance	October	340
1946 January	Warren closure	November	171
February	238	December	563
March	400		
April	1,200	Totals	106,268

For purposes of comparison, the average number of *Bulinus* caught on the traps per week per km. was calculated for each canal (Table XI) and also for each month (Graph). It will be seen that the average catch of snails in 1945 varied from a half to 224 snails per week per km. in different canals, the average being 37.5 snails. In 1946 the variation had been reduced, not exceeding 66 snails per week per km., and the average weekly catch per km. for all streams had fallen to 12 snails.



GRAPH.—Showing the number of *Bulinus* collected per week per kilometre by means of palm-leaf traps in main canals, Fayoum Province.

Although the work in general has been intermittent and the data not wholly representative owing to sulphations from December 1945 to May 1946 and again in November to December 1945 it is quite evident from the records

TABLE XI

RESULTS OF TREATMENT BY PALM-LEAF TRAPS IN THE FAYOUM, JULY, 1945, TO
DECEMBER, 1940

Name of canal	Length in km	<i>B. truncatus</i>		
		Total collected	Average per km	
			1945	1940
Bahr Youssef	24 100	13,575	11 4	0 5
" Wassef	13 200	1,734	1 0	3 1
Teret el-Agouz	0 500	5,810	22 3	9 0
" Hawaret Adlan	0 300	7,084	32 7	11 2
" Wahby	08 120	1,723	0 46	0 40
Bahr el Adawa	6 610	5,656	50 3	15 3
" el A'lam	6 174	4,135	27 4	10 5
" el Maslûb	0 919	1,905	44 0	97 9
" Dar el Ramad	0 540	5,506	10 9	35 2
" Sennoures	10 504	5,275	0 3	14 4
" Tanhala	10 490	4,244	1 8	11 3
" el Zawiya	7 100	2,727	34 5	0 0
" Talet el Ali	9 000	2,000	9 6	3 1
" " Wati	10 500	5,739	13 3	10 1
" el Wastani	4 000	2,715	00 8	4 0
" el Ekta wa Garadu	16 500	2,975	7 0	3 1
" Dissin el Gedid	4 750	2,024	10 3	12 0
" Tobhar el Ali	5 500	23,991	217 2	20 2
" " Wati	4 000	2,622	25 2	9 0
" Arouss	11 000	5,817	30 1	10 4
" el Nazla	30 215	2,368	0 90	1 2
" el Gharaq el Omûmi	28 525	3,243	0 9	2 3
" Seif el Din	5 395	10,818	115 3	5 2
" Awlad Mohamed	4 665	1,705	19 5	3 0
" Qualamsha	3 350	4,904	56 7	18 1
" Abou Dinguash	7 458	1,211	7 5	7 9
" Asr el Banât	24 870	902	1 3	0 0
" El Bashawât	22 286	106,284	224 1	7 6
" Abgig el Ali wa el Wati	5 700	1,762		18 1
" Ahriet wa el Atamna	7 580	996		11 0
" Aboqsah	11 800	401		2 4
" Abshawai	15 000	223		1 1
" El Horishi	4 300	250		8 6
" Anz	5 500	312		9 4
Gannabiet Anz	2 300	248		21 5
Bahr Matûl	2 160	75		17 8
" Nakalifa	3 460	197		7 2
" Abo el Mir	10 050	237		3 0
" el Ghaba	12 860	207		2 7
Totals	454 087	245,216	37 44	11 76

that the number of snails collected in the first 2 or 3 months fell sharply never again to reach figures approaching those of the first collection. Although these results are in part due to sulphation and seasonal variation, it is believed that whenever the treatment of canals infested with snails is not possible, due to difficulties in closing the intakes, or on account of the lack of labour in a given area, palm-leaf traps can be very effectively used as an alternative method in the control and reduction of the snail population of the streams. They are also very useful in the survey of large streams, especially when these are only lightly infested with snails.

SUMMARY

Palm-leaf traps have been used by the Bilharzia Destruction Section of the Egyptian Ministry of Public Health for some time in surveying streams for *Bulinus truncatus*. The method is superior to survey by dip-net in streams with a scanty snail population.

The palm-leaf trap is also recognized as a cheap and regular method for snail removal which is especially convenient when clearance and sulphation of streams is not possible owing to difficulties of water control.

An account is given of the application of palm-leaf traps both in survey and snail removal.

A series of experiments is described which throws light on the extent to which snails are carried by large streams and at what levels the proportion of snails caught by traps in the presence or absence of other vegetation and the distribution of snails in streams under various conditions of current and depth.

It is concluded that a small number of snails are carried in the middle and upper layers of water in the main canals that a large proportion of *Bulinus* present in the stream can be caught with palm leaves, even in the presence of other aquatic vegetation and that the snails are distributed equally over the bottom and slopes of the canal bed in shallow canals but are more or less confined to the slopes in deeper and swifter canals. They do not withstand a strong current.

AN ATTEMPT TO CONTROL HOUSE RATS IN RANGOON

BY

J L HARRISON, A R C S , M S C *

AND

H C WOODVILLE, A R C S M S C

Three years of bombing and of Japanese military administration had caused such a degeneration of the sanitary condition of Rangoon that, at the time of the Allied reoccupation in 1945, special arrangements were made to deal with a possible outbreak of bubonic plague. The success of the prebaiting method of poisoning *Rattus norvegicus* suggested that this method should be tried against the rats met with. This is an account of the method evolved and its success during the period May to November, 1945. An account of the investigations needed to establish the method is being published elsewhere.

Five species of rodent and one insectivore infest the houses of Rangoon. They are —

Muridae (Rodentia) *Bandicota bengalensis* (GRAY and HARDWICKE), *Rattus norvegicus* (BERKENHOUT), *R. rattus* (LINNÉ), *R. exulans* (PEALE), *Mus musculus* (LINNÉ)

Soricidae (Insectivora) *Suncus caeruleus* (KERR)

The relative abundance of these six species is indicated in Table I, which shows the total number and proportion of each species to be brought into the laboratory. Since all specimens trapped or poisoned in various areas at various times were brought for examination, these figures should be reasonably unbiased. The maximum and minimum proportions of each species from different areas in which not less than 100 were obtained is given also.

* Our thanks are due to the Medical Officers and Inspectors of the Health Department of Civil Affairs Staff (Burma) and of the Rangoon Municipality for their willing co-operation, to U KAW THEIN and U TUN NGWE, for their permission to use parts of the Zoological Gardens, and to the members of No. 1 Pest Destruction Advisory Unit, R.A.S.C., and U MYO KHIN, B.Sc., and MAUNG GALE, B.Sc., for their assistance at all times.

TABLE I.

NUMBERS OF DIFFERENT SPECIES BROUGHT TO LABORATORY IN MAY TO NOVEMBER, 1943 AND RANGE OF PROPORTIONS FROM DIFFERENT LOCALITIES FROM WHICH NOT LESS THAN 100 SPECIMENS WERE OBTAINED.

	Number	Per cent. of total.	Maximum and minimum per cent. in different localities.
<i>Bandicota bengalensis</i>	863	31	14-56
<i>Rattus norvegicus</i> ...	167	6	0-16
<i>R. rattus</i> ...	212	8	1-17
<i>R. exulans</i> ...	1,246	45	4-78
<i>Mus musculus</i>	144	5	1-8
<i>Sorex cervinus</i>	123	4	11
Total	2,765		

In the following notes on the species size ranges are those of approximately 95 per cent. of all adults. The information given and abbreviations used, are —

W = Weight in grammes.

L = Length of head and body (nose to anus) in mm.

T = Tail length (anus to tip) as percentage of head and body length.

HIF = Length of hind foot (excluding claws) in mm.

M = Mammæ as number of pairs f pectoral and number of pairs of pelvic = total number

Further details are deposited in the University of London M.Sc. Thesis (Woodville, 1947)

Bandicota bengalensis the "Indian Mole Rat" or Lesser Bandicoot Rat = *Neobala* sp. = *Gomomys* sp. "Gomomys leek, the name often used for the Indian form, is either synonym or subspecies.

W 50 to 600 g; L 130 to 260 mm. T 75 to 95 per cent. HIF 30 to 45 mm
M variable 12 to 20 not always symmetrical.

The muzzle is blunt and the ears small. The fur is coarse mixture of black and brown hairs giving the appearance of dark grey-brown above grey below usually dirty enough to soil the hands. This species can be confused only with *Rattus norvegicus*. A final distinction, all pads on the hind feet are rounded whereas in *Rattus* spp. the pads at the base of the outer toes of each hind foot are heart- or kidney-shaped, usually with small accessory pad.

Adults of both sexes had characteristic snell reminiscent of pigs. When trapped or cornered they erected their bristles and spat rather like cat at other times and when working hard, they made piglike grunting. They appeared to be incapable of limbing, but were formidable burrowers with great powers of penetration through even poor quality concrete. The burrow were extensive with an average of about ten entrance holes.

Rats of this species were abundant in all parts of Rangoon. They entered houses and stores at ground level, and made burrows in the earth floor of poorer houses.

Rattus norvegicus the cosmopolitan brown rat = *R. decussatus* (Pallas)
W 50 to 375 grammes L 125 to 250 mm. T 80 to 115 per cent. HIF 30 to 45 mm. M 3 + 3 = 12.

The muzzle is longer and sharper than in *Bandicota*, the ears rather larger, the fur is less coarse, grey brown above, grey below

Rats of this species were confined to the docks and central town areas. In Kantawgale district of Rangoon they were not found north of Little Sisters road, and none were found in Bahan, the plague centre

Rattus rattus W 40 to 160 grammes, L 110 to 200 mm, T 95 to 135 per cent, HF 25 to 35 mm, M $2 + 3 = 10$ or $3 + 3 = 12$, sometimes with 11 mammae

The common form has a reddish-brown back and a sharply defined white belly, evidently belonging to what appeared to be the cline *R. r. khyensis* Hinton (N Burma)—*R. r. jalorensis* (Bonhote) (Malaya). A very few nondescript specimens of the ship-rat type were taken. The typical form was unmistakable, the nondescript could be confused with *R. norvegicus* from which long tail, sharper nose, and larger ears distinguished it, or with *R. exulans* from which size and mammae distinguished it

These were decidedly tree rats. We have observed colonies running about in the branches of trees and bamboos and entering houses through the roof. They did live inside houses, where they were responsible for damage to clothing and books. In contrast to *Bandicota*, specimens of this species were always very clean

This species was present in all parts of Rangoon, particularly in better-class houses, but nowhere was it the most abundant species

Rattus exulans, the "Little Burmese House Rat" = *R. concolor* (Blythe). *R. exulans concolor* is the westernmost representative of a species which ranges right across the Pacific Islands, and which was first described from Fiji under the name "*exulans*"

W 15 to 60 grammes, L 85 to 140 mm, T 100 to 140 per cent, HF 20 to 25 mm, M $2 + 2 = 8$

This rat is like a small *R. rattus*, but the colour a greyish brown with the underside grey, never white. The number of mammae is constant and characteristic

In habits this species strongly resembled *R. rattus*, and indeed the two species often occurred together. *R. exulans*, however, was more abundant inside houses than out

This species was the most abundant in all parts of Rangoon. These rats, however, were less conspicuous than *R. rattus*, and in mixed colonies the white bellied species was the more often described by householders

Mus musculus the cosmopolitan "House Mouse"

W 7 to 25 grammes, L 60 to 100 mm, T 95 to 135 per cent, HF 13 to 22 mm, M $3 + 2 = 10$

This animal was not always easy to distinguish from *R. exulans* except by mammae. Although present everywhere, it was nowhere abundant

Suncus caeruleus the "musk shrew" or "muskrat" = *Crocidura* sp. = *Pachyura* sp., probably = *S. murinus*

W 20 to 100 grammes, L 90 to 150 mm, T 50 to 80 per cent, HF 17 to 25 mm, M $0 + 3 = 6$

The nose is long and mobile, the teeth more uniform, without chisel-shaped incisors, the eyes are very small, about 1 mm in diameter, the tail very thick and sparsely haired, the fur is soft and smoky grey in colour

All specimens had a characteristic musty smell and a very shrill squeak

This shrewmouse was abundant in all parts of Rangoon. It was carnivorous, although it would take a variety of foods from biscuits to bully beef, it probably lived on insects such as cockroaches and crickets, although it would eat young rats readily. Table I gives a false estimate of its abundance because it was not affected by poison campaigns and therefore very few bodies were obtained except by trapping

Rats are of importance for two reasons, damage and disease. Whilst in Rangoon they were more feared as carriers of disease, it is probable that were the importance of the damage fully realized it would be considered to outweigh the question of disease

In Rangoon rats were encouraged. The staple food of the human population was rice, and no one seemed satisfied with his meal unless some rice was

left over at the end. Although the civil authorities endeavoured to have the rubbish dumped in one place and cleared, much of the rice was scattered around the inhabited areas to be eaten by dogs, crows, and rats. In such circumstances, a large rat population was maintained. Rats were being trapped at the rate of some 700,000 a year and in the course of a year 700,000 rats will eat over 2,000 tons of rice.

BUBONIC PLAGUE.

Rangoon was reoccupied by Allied troops in May 1945. Cases of bubonic plague were appearing in the Bahan area of the town, but owing to poor diagnosis and notification at first, the size of the outbreak was not realized. By the end of June it was realized that plague was assuming dangerous proportions, and a mass poison campaign was commenced in this area. The first treatment took a month and after that only one outbreak of three cases was recorded up to the time of leaving the town in November. In all, 11 cases were notified from Bahan itself but it is certain that many were missed.

All rats brought into the laboratory not needed for experiments were killed and examined for fleas and plague. For plague spleen of spleen was examined. Suspected smears were stained by gram stain, and if gram negative were passed to an army pathological laboratory.

The results of all examinations are shown in Table II. It will be seen that in only three rats were plague bacilli found, all three were obtained from Bahan, the two *R. rattus* at the end of July the one *Banahote* on 10th August. One of the two *Rattus* was picked up dead in the house of a plague casualty the other two rats were trapped.

TABLE II.
RATS EXAMINED FOR PLAGUE, JULY TO NOVEMBER, 1945.

	Bahan.		Elsewhere.	
	Number examined.	Plague +	Number examined.	Plague +
<i>Bombus terrestris</i>	75	1	493	0
<i>Rattus norvegicus</i>	0		124	0
<i>R. rattus</i>	41	2	104	0
<i>R. exulans</i>	127	0	429	0
<i>Mus musculus</i>	17	0	21	0
<i>Suncus coronatus</i>	18	0	60	0

On two other occasions the investigation of plague casualties produced reports of white bellied rats falling dead from the rafters. Investigation of reported infestations by white bellied rats showed that such infestations

were usually mixed colonies of *R rattus* and *R exulans*. As noted above, *R rattus* is the more conspicuous, and it is possible that reports of "white bellied" rats dying in numbers may really include both species.

Fleas—Only the two species of *Xenopsylla* were found. Identifications made from the keys in SMART (1943) were kindly confirmed for 473 of its fleas by Major LEESON, of the London School of Hygiene and Tropical Medicine.

TABLE III
MEAN NUMBERS OF FLEAS PER RAT OF ALL LIVE RATS EXAMINED

	Number examined	<i>Xenopsylla astia</i>	<i>Xenopsylla cheopis</i>
<i>Bandicota bengalensis</i>	521	1.45	0.14
<i>Rattus norvegicus</i>	121	0.56	0.33
<i>R rattus</i>	111	0.23	0.30
<i>R exulans</i>	387	0.27	0.29
<i>Mus musculus</i>	28	0	(1 flea only)
<i>Suncus caeruleus</i>	87	0.07	0.21

There were no noticeable differences in the distribution of fleas in different areas of the town. Further details of flea distribution are deposited in the University of London, M Sc Thesis (HARRISON, 1947).

Important Species

From the lack of *R norvegicus* in Bahan where the plague outbreak was centred, it was evident that this species was not involved. *M musculus* was ruled out by lack of suitable fleas, and it appeared likely that since *B bengalensis* bore so few *X cheopis* it too was of little importance. The inference, therefore, was that plague was carried by *R rattus* and *R exulans*, and the occurrence of dead infected *R rattus* pointed to this species as the most important. This evidence, however, was not sufficient to implicate any one species to the exclusion of others and control measures were directed against all the rodents. In the absence of any evidence to implicate *S caeruleus*, this shrew was left unharmed.

METHODS OF CONTROL IN USE

The danger of plague had been sufficient to justify a permanent organization for the control of rats in Rangoon. Under the health department, the town was divided into a number of areas each under an assistant medical officer of health. In each area a sanitary inspector supervised rat control, which was carried out by one or more gangs of coolies. Each gang consisted of a charge hand and about six coolies who, in 1945, were receiving Rs 1/2 as and 14 as a day.

respectively. As can be expected, the grade of labour obtained for this wage was low. Control work consisted of trapping and gassing.

Trapping

Wooden cage traps were used, each of internal dimensions about $14 \times 14 \times 25$ cm. These traps worked on the principle by which taking of the bait released a door which closed either by a spring or under its own weight. Both patterns were of a hard teak like wood and almost totally enclosed, the maximum aperture being a small barred window at the end or in the roof.

Traps were laid in a few streets at a time. Usually two traps were given to each householder who was expected to bait and set them. Rats were collected on the following morning. Trapping was continued in each area for from 4 days to a week or more. When, at our instigation, detailed records were kept no significant change in the number of rats caught each night could be observed even when trapping was continued in the same area for some weeks (e.g., Fig.). The average catch during the whole period observed was 4.5 rats per 100 traps per night. This figure is much less than that given by the Harcourt Butler Institute (1939), who obtained 7.5 per cent. per night, but with no other method of control in operation.

Trapping had been the popular method of "control" for many years. The Harcourt Butler Institute Report (1939) records that the total number of rats trapped in Rangoon during 1934-38 were: 1934 734,639 1935 709,632 1936 753,618 1937 718,467 1938 569,590—an average of about 2,000 a day.

Trapping was enforced under the Japanese, and reports indicate that this figure was maintained. Household-ers were responsible that rats were caught. A catch was rewarded with ten cents and a lottery ticket, a failure brought the punishment of being carried by lorry for some miles into the country and being left to walk home. The chief result of such enforcement was a trade in live rats to put into the traps.

Rats had been removed from the Rangoon population at a rate of some 2,000 a day for many years, and yet the rats were, to all appearances, flourishing. It was evident that trapping as a means of control was of little value.

Gassing

The method of pump gassing was much favoured by the local authorities. Our experience of the method was not favourable.

The method consisted of pumping down one of the rat burrows a cloud of dust which released hydrogen cyanide in contact with air or soil moisture. Other holes were blocked as clouds of dust appeared from them and finally the hose was withdrawn and the hole blocked. Powders used were "cyanogen A" and "cyanog" our experience was with "cyanog" which is reputedly less efficient for the pumping method. The spoon method was not used owing to difficulty of finding all holes.

The efficiency of gassing was found to be low. A gang of four to six coolies, with one pump, could deal with up to 400 holes a day. Usually about 40 holes were gassed and the rest blocked. In Rangoon the number of holes per unit area was often very large, thus one gang under a British corporal took a month to deal with an area of about half a square kilometre, which was not unusually heavily infested.

The kill appeared to be poor, large numbers of holes reappearing after gassing. Thus in one market place all visible holes, 231, were gassed, a week later the area was gassed again and 173 holes were found, on the following day 20 fresh holes were found.

With the exception of the limited area where *R. norvegicus* occurred, nearly all rat burrows seemed to be the work of *B. bengalensis*, all dead rats obtained by gassing operations were of this species.

To investigate the reasons for repeated failure, an attempt was made to measure the concentration of hydrogen cyanide in the burrow after gassing. A length of lead capillary tubing was inserted about 60 cm down a hole which was gassed and blocked as part of the usual routine. Fifty ml samples of air were withdrawn at intervals and HCN estimated. The method was considered to give a figure accurate to about 0.3 mg per litre. Results are shown in Table II, and safety limits for humans (HENDERSON and HAGGARD, 1943) given for comparison.

TABLE IV
CONCENTRATION OF HCN IN *B. bengalensis* BURROW AFTER PUMP-GASSING WITH
"CYMAG," AND SAFETY LIMITS

Time after closing hole	Concentration of HCN	
	Mg per litre	Parts per million
0	2.4	2,000
45 mins	2.4	2,000
80 "	1.2	1,000
100 "	0.9	750
120 "	0.3	250
23 hours	less than 0.3	less than 250
Rapidly fatal	3.6	3,000
Fatal after 30 mins	0.6	500
Safe for 60 mins	0.06	50

The concentration achieved in the main burrow was just sufficient, but remembering the branching system of burrows with many blind alleys, it can be assumed that a sufficient concentration was not likely to be obtained in all parts of the burrows.

In our view therefore, gassing was of doubtful value against *B. bengalensis* and since the principal plague carrier seemed to be *R. rattus* which did not live in burrows, gassing was of no value in combating plague.

Poisoning

As far as could be discovered, poisoning had never been used in Rangoon. At the time a fairly large supply of barium carbonate of various grades was available, but no other poison except sodium arsenite which, as tests confirmed, was unacceptable. Some white arsenic arrived later but not until just before our departure.

The method of prebaiting and poisoning had been used in Hawaii by DOTY (1938 and 1945) and had been worked out in detail for *R. norvegicus* and *R. rattus* in Britain by the Bureau of Animal Population, Oxford University (not yet published), and the British Ministry of Food Infestation Control (1946). The method consisted of conditioning rats to feed on a bait at certain points. When the rats in the colony were effectively conditioned the bait was replaced by the same material mixed with a suitable proportion of poison. If any rats were left this poisoning was followed up using a different bait and a different poison. This technique properly used was found to kill all the rats in the colony. It was decided to try out this method against the Rangoon species.

Bait Base.—Tests showed that both individuals and colonies of *B. bengalensis* and of *R. rattus* and *R. exulans* showed a marked preference for rice over any other foodstuff tested. Boiled rice was preferred to dry rice, and since the amount eaten appeared to depend on the dry weight (24 per cent.) the bulk eaten was large. Boiled polished rice was thus a very convenient bait and poison base since it was bulky powder stuck to it readily and there being no husk the whole grain was eaten. It was found to attract rats accustomed to feeding on other food, and with unprejudiced rats even poisoned rice was preferred to other foods. Boiled rice, therefore, was adopted as the standard bait.

Poison.—Barium carbonate, the only poison available, is of low toxicity. The dose necessary for certain kill seemed to be of the order of 1 gramme per kg. body weight. It was therefore very fortunate that the rats accepted a bait of such large bulk as boiled rice. Tests with rats conditioned to feed on flour showed that a sufficient dose of barium carbonate could not be taken in such a bait.

Admixture of even a small quantity of barium carbonate was found to produce a marked reduction in the amount of foodstuff eaten. Increase of the proportion caused a progressive reduction of take until with more than about 25 per cent. little or no bait was eaten. The maximum amount of barium carbonate was ingested when boiled rice contained about 14 per cent. of it. To allow for uneven mixing the standard poison mixture adopted was 18.7 per cent. or one part by weight of barium carbonate to five parts of boiled rice.

The result of a large number of tests and calculations showed that when a mixture of this sort was used the amount of barium carbonate ingested by the rats was between 1 and 10 grammes per kilogram body weight. This allowed only a small margin for error or abnormal feeding.

Prebaiting—Tests indicated that *B bengalensis*, *R rattus*, and *R exulans*, showed the same avoidance of new objects and of new foodstuffs as had been found with *R norvegicus* and the cosmopolitan forms of *R rattus*. They also showed a similar preference for feeding at the same place and on the same foodstuff each night. As a result of these and other factors, when a new bait was laid little was taken at first and thereafter the take increased daily. The maximum take was reached in about 4 or 5 days and thereafter the take usually oscillated about this maximum.

If the bait had been poisoned at any time before the maximum take, some or all of the rats would have failed to eat their fill at the baiting point. As noted above, the margin for error was small and therefore a large proportion of the rats would have taken a sublethal dose. For this reason poison bait was not laid until the fifth night of baiting, and care was taken to lay the baits in the same place each day.

Poison Shyness—Some tests were made on rats which had survived a sublethal dose of poison. Results varied, but in general either they would not accept barium carbonate in rice a second time within a month, or if they did accept they showed a marked resistance to the poison.

Procedure—The procedure adopted was

- 1 Baits of from 4 to 8 ounces (100 to 200 grammes) of boiled rice were laid at suitable points
- 2 Rice was renewed daily for the 4 days of prebaiting
- 3 On the fifth day each bait was replaced by a similar amount of boiled rice containing about 16 per cent (1 in 5) barium carbonate
- 4 On the sixth day excess poison bait was collected and destroyed, dead rats were collected

Containers—To facilitate the finding, collecting, and replacement of baits, and to protect them from rain, dogs, and trampling, it was found of advantage to put each into a covered container. One promising container was a short section of large diameter bamboo, but because of the large number of traps available and already familiar to householders the container adopted was the standard wooden trap with the sliding door removed. The dose of prebait or poison bait was put into the traps which were then distributed to baiting points. Tests showed that after the initial avoidance the boxes were visited readily by the rats.

Records—Baits were examined daily and the result of each recorded as "Complete," "Take," or "No take," according to whether its rice had disappeared, been partly eaten, or appeared untouched. Such records proved of value in reviewing the progress and efficiency of poisoning.

Dangers—The usual objection made to poisoning methods is that they

are dangerous to humans and other animals. During this campaign no human casualties were recorded and no claims were made for loss of domestic animals. Except in some private premises, all baits were laid in the containers mentioned above, and the prebaiting gave ample opportunity to warn everyone concerned. The bait was of such consistency that it had to be eaten on the spot, and in grain stores the use of the bait container ensured that all residues were collected and not left to be swept up and rebagged with the spilt grain.

In these circumstances no danger was to be expected, but at first poison baits were dyed with methylene blue by adding a concentrated solution to the water in which the rice was boiled. Although this gave a satisfactory colour it was disliked by the operators and later abandoned as unnecessary. Dyed baits, however, were used occasionally in exposed positions.

Control by Poisoning

In Rangoon the object was to prevent the spread of the bubonic plague outbreak which was occurring in the Bahan area. In this region rats were so numerous as to constitute an apparently continuous population, conditions, presumably favourable to the spread of plague. It was decided, therefore, to try to reduce this population quickly over a large area rather than to attempt local extermination.

Poisoning was carried out in areas of about 100 houses at a time. Rice was boiled centrally and distributed in containers to the householders. Two or three containers were given to each householder who laid them where he thought best. The baits were replaced daily and the take from each recorded. It is feared that some "complete" takes were caused by the householders throwing away the bait.

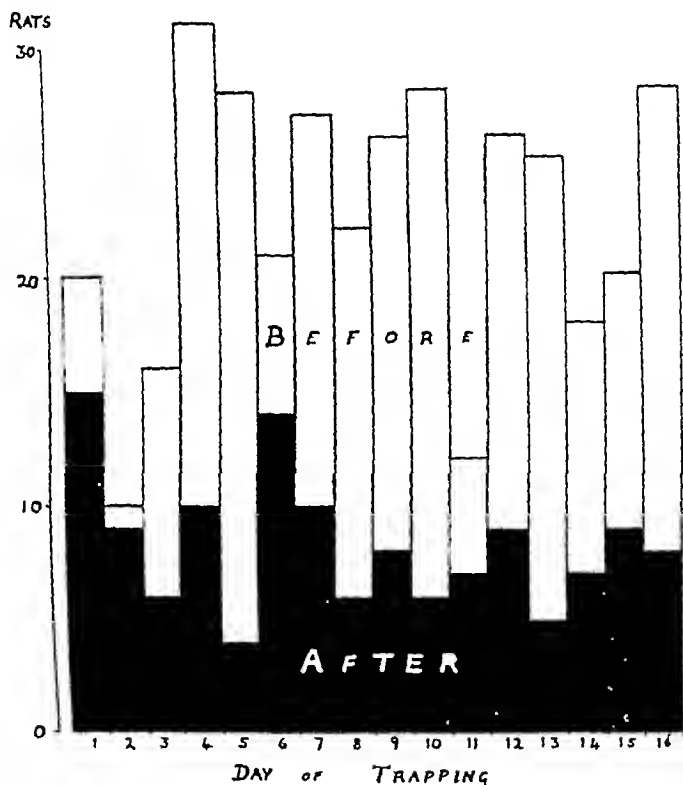
Poisoned rice was laid on the fifth night of baiting. On the following day all containers were collected, takes recorded, excess poison burnt or buried. Dead rats were collected at the same time, but the search for bodies was not rigorous.

Success in rat control is indicated not by dead rats, but by the absence of live ones and their damage. Thus the number of dead rats found and the amount of poison taken may not be used as measures of success. In this instance success was estimated by absence of plague and reduction in number of rats.

In Bahan, after the poison campaign was well under way in July the only cases of human plague were three occurring at the same time and place in late August. None other was recorded up to the time of leaving in November. Past records, however, suggest that in Rangoon this disease reaches its maximum in July or August and thereafter dies away so such a result would not have been unexpected without any control of rats.

The results of trapping were so variable that no reliance could be placed on the trapping rate except in identical circumstances. For only one area were such accurate and comparable records available before and after poisoning. In

91st to 94th Streets, Kantawgale, trapping was carried on continuously for 16 days up to 28th August using approximately 323 traps per night. This area was poison baited in two parts on 6th and 12th September. On the 15th September trapping was renewed for a further 16 days using approximately 320 traps. The number of rats caught each night is shown on the histogram.



Histogram of the number of rats trapped each night in 91st to 94th Streets, Kantawgale, during two 16-day trapping periods, one before poisoning and one after. Approximately the same number of traps was used each time.

The reduction in number trapped was marked. Before poisoning the mean number trapped was 22.4 per night with a standard error of mean of 1.5, after poisoning the mean number trapped was 8.3 (s.e. 2.2) that is a reduction of about two-thirds.

DISCUSSION

It is evident that poisoning can be used safely and effectively in Rangoon. It is true that in this campaign only a 60 per cent kill was obtained over a large area, but, as has been pointed out, local extermination was not attempted and

a low efficiency poison was being used. Even so, the method was much more effective than either trapping or gassing. By using a suitable sequence of baits and poisons, by achieving a reasonable improvement in sanitation, and by organising a systematic block control, it should be possible to reduce the rat population of Rangoon to a small fraction of its former size.

Further research is needed on food preference and on the acceptability and dosage of various poisons so that a suitable sequence of baits can be determined. Preliminary tests, however should not take long and detailed investigation could be combined with active control.

SUMMARY

1. An outbreak of bubonic plague was expected at the time of the Allied reoccupation of Rangoon in 1945. Rat control by poison was attempted.

2. The town was infested with *Bandicota bengalensis*, *Rattus rattus*, *R. exulans*, some *R. norvegicus*, *Mus musculus*, and the shrew *Suncus caeruleus*. Notes on the appearance and habits of each are given.

3. Plague seemed to be associated with the first three only.

4. Trapping and gassing methods had long been in use and were considered ineffective.

5. A method of poisoning with barium carbonate in boiled rice after 4 days prebaiting was evolved.

6. Results were satisfactory and with further research the method should prove an effective method of control.

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SEROLOGICAL EXAMINATION OF HUMAN AND CATTLE SERA FROM PALESTINE FOR THE PRESENCE OF ANTI- BODIES AGAINST A BOVINE STRAIN OF *LEPTOSPIRA* *

BY

H BERNKOPF,
L A STUCZYNSKI,
T GOTLIEB
, AND
CH HALEVY,†

Department of Hygiene and Bacteriology, The Hebrew University, Jerusalem

Leptospirosis in cattle is a disease which has only recently been recognized in Palestine (BERNKOPF, OLITZKI and STUCZYNSKI, 1947). A few localized outbreaks have been observed and its symptoms have been described, but the actual frequency of the disease in this country is unknown. Yet such knowledge is desirable, if only for the reason that the same agent which causes the cattle disease is also responsible for severe human leptospiral infections. About 40 human cases of leptospirosis have been diagnosed in Palestine during the last 2 years, at least ten of the patients being butchers.

The diagnosis of the bovine disease is not difficult if it occurs in its severe and often fatal form, which is characterized by jaundice, nephritis, haemolytic and haemorrhagic symptoms. But animals with the more frequent milder forms without jaundice easily escape detection, though they constitute a graver danger, from the epidemiological point of view, than severely jaundiced animals. Laboratory diagnosis must rely on the demonstration of the aetiological agent or of specific antibodies in the sera of the diseased animals. In addition to its

* This work was aided by a grant from the Palestine Board for Scientific and Industrial Research.

† We acknowledge with gratitude the help and co-operation of many physicians and veterinary surgeons, particularly among them Mr G B SIMMINS, Director of Veterinary Services, Dr R S F HENNESSEY, A/D Medical Services, Dr Y S GOOR, Veterinary District Officer, Tel-Aviv, Dr G E AJOUB, Medical Officer, Jaffa, Drs S BRESH, K MARBERG and E E LEHMAN, Medical Officers, Government Hospital, Tel-Aviv, Dr A LEVITT, Slaughterhouse, Tel-Aviv, Dr A BIRMAN, Slaughterhouse, Jerusalem, Dr B KIRSCH, Slaughterhouse, Jerusalem, Dr S DREIFUS, Herzlia, Dr A MARKIN, Kfar-Saba, Drs H HELLER, CH SHIBER, J CASPER and L REIF, Beilinson Hospital, Petach-Tikvah, Dr H NATANSON, Haifa, Drs W GOTTFRIED and D BURSTEIN, Raananah, Dr V ELLENBOGEN, Kfar-Malal.

simplicity the serological method has the advantage of making a retrospective diagnosis possible, since the antibody titre in the serum remains high for a number of years in many cases of leptospiral infection. It was therefore decided to use the agglutination test for the examination of the blood of cattle in public slaughterhouses to gain an approximate picture of the frequency of the infection.

TECHNIQUE.

About 5 c.c. of blood was obtained from each animal when it was killed in the slaughter house, and the blood was brought to the laboratory in sterile centrifuge tubes. After centrifuging the unheated serum in dilutions of 1:20 and 1:200 was employed for the agglutination tests. Formalized, concentrated suspensions of the bovine strain of *Leptospira* isolated in this country which is closely related to *L. grippityphosa* (BENNETT and STUCKENREIN, GOTTLIEB and HALPERY 1947) and of a strain of *L. icterohaemorrhagiae* (the Jackson strain, kindly supplied by Dr J. C. BACON, The Wellcome Laboratories of Tropical Medicine, London) were used as antigens. Serum dilutions and leptospira antigen were mixed in equal quantities in small test-tubes and were incubated at 37°C. for 2 hours. The tubes were then kept at room temperature overnight. Results were read the following morning by examining uncovered drops by dark field illumination with low power objectives. Sera causing positive reactions in dilutions of 1:200 were always retested, and the highest dilution giving unequivocal agglutination was determined.

SEROLOGICAL EXAMINATIONS OF SERA FROM SLAUGHTER CATTLE.

During the course of one year (1946 to 31.5.47) the sera of 869 head of cattle brought to the slaughterhouses of Jerusalem, Tel Aviv, Hertsah and the Kfar-Saba district were examined for agglutination against *Leptospira*. Fifteen batches of sera (11 to 174 sera samples in each) were tested at different times of the year. Data on the agglutinations observed at each slaughterhouse are presented in Table I.

TABLE I
DISTRIBUTION OF CATTLE SERA ACCORDING TO SLAUGHTERHOUSE.

Slaughterhouse	Total of cattle examined	Pos- -tive 1:20	Pos- -tive 1:200	Local origin	Pos- -tive 1:20	Pos- -tive 1:200	Foreign origin	Pos- -tive 1:20	Pos- -tive 1:200	Origin undeter- mined	Pos- -tive 1:20	Pos- -tive 1:200
Jerusalem	264	33 (19)	33 (9.9)	170	3	9	189	7	11	25	1	3
Tel-Aviv	544	91 (16)	49 (9.0)	144	16	17	228	34	70	174	41	13
Kfar-Saba Hertsah	91	29 (47)	11 (12)	97	29	10	4	—	1	—	—	—
Total	869	153 (17.9)	93 (9.3)	311	48 (14.9)	36 (11)	320 (12)	41 (12)	82 (9.4)	209	42 (7.0)	15 (1.7)

Of the 869 sera examined, 153 (17.6 per cent) gave a positive agglutination reaction in a dilution of 1:20, and 83 (9.5 per cent) in a dilution of 1:200. Positive sera were encountered in every batch though their percentage varied with each group of sera.

Thirty-six sera with a positive reaction in a dilution of 1:200 from seven batches (450 sera in all) were titrated with both the bovine strain of *Leptospira* and with *L. icterohaemorrhagiae* to determine their final titre. The results of this series of examinations are given in Table II.

It will be seen that among 36 sera so examined, two were still positive in a dilution of 1:3,200, nine in a dilution of 1:1,600, nine in a dilution of 1:800, and eight in a dilution of 1:400 and 1:200, respectively. With the exception of one serum, which reacted with both strains to a titre of 1:400, all these sera reacted either negatively or in a lower titre with *L. icterohaemorrhagiae*. It is particularly noteworthy that the two sera with a titre of 1:3,200 against the bovine strain reacted with *L. icterohaemorrhagiae* in dilutions of only 1:20. Among nine sera positive against the bovine strain in a dilution of 1:1,600, four were completely negative against *L. icterohaemorrhagiae*, three were positive to a titre of 1:20, and only two were positive against *L. icterohaemorrhagiae* in dilutions of 1:200 and 1:800, respectively. Infections of cattle with *L. icterohaemorrhagiae*

TABLE II

COMPARISON OF AGGLUTININ TITRES OF 36 POSITIVE SERA AGAINST *Leptospira icterohaemorrhagiae* AND THE BOVINE STRAIN OF *Leptospira*

Maximum titre against the bovine strain of <i>leptospira</i>	Number of positive sera	Number of same sera positive against <i>Leptospira icterohaemorrhagiae</i> in dilutions of						
		Negative.	1:20	1:200	1:400	1:800	1:1,600	1:3,200
1:3,200	2	—	2	—	—	—	—	—
1:1,600	9	4	3	1	—	1	—	—
1:800	9	2	2	2	3	—	—	—
1:400	8	4	1	2	1	—	—	—
1:200	8	3	5	—	—	—	—	—

are reported in the literature (KATHE, 1943). Concurrent infection with *L. icterohaemorrhagiae* and the bovine strain cannot, therefore, be excluded, but it seems probable that these reactions, nearly always of a lower titre against *L. icterohaemorrhagiae* than against the bovine strain, may be regarded as coagglutination reactions. The following fact also supports this point of view: several young calves which were experimentally infected with the bovine strain developed an agglutination reaction not only against the bovine strain but also against *L. icterohaemorrhagiae*, though to a much lower titre than against the homologous strain.

With no previous survey of this sort to serve as a precedent, the question arose which serum titre should be regarded as indicative of a past leptospiral infection. The possible occurrence of "natural" antibodies against *Leptospira* in bovine sera must be borne in mind. A serum titre of 1:200 or higher was considered significant for the following reasons: (1) The sera of cattle from farms under the strictest veterinary control in which no cases of leptospirosis had occurred, were always negative in dilutions of 1:200, whereas positive agglutination reactions with the bovine strain and with *L. icterohaemorrhagiae* sometimes occurred in such cases with dilutions of 1:20. Sera of young calves

were always completely negative against both strains. (2) The agglutinins in sera with a positive agglutination reaction in a dilution of 1:200 were specific for the bovine strain. When tested against *L. icterohaemorrhagiae* these sera were either negative or, in general, reacted in a lower titre. (3) Sera which were positive with the bovine strain to a titre of 1:200 were frequently positive in higher dilutions as well. (4) A serum titre of 1:200 is generally considered to be proof of a past infection with *L. icterohaemorrhagiae* in human beings and in rats. (5) Non-specific antibodies frequently disappear after heating the sera for 30 minutes at 56° C. As will be seen from Table III, four positive sera which were treated in this way either retained their previous titre, or showed only a slight decrease.

If dilution of 1:200 be accepted as proof of past infection with the bovine strain, then as high percentage as 9.5 per cent. (83 among 869 animals) must be regarded as

TABLE III.

INFLUENCE OF HE. ON THE AGGUTINATION REACTION IN POSITIVE SERA.

Serum number	Titre against bovine leptospira strain of fresh serum.	Titre against bovine leptospira strain of serum after inactivation (30 minutes, 56 C.).
1	1:400	1:400
2	1:800	1:800
3	1:400	1:400
4	1:800	1:200

having been infected in the past. But even if minimal titre of 1:1600 were to be regarded as significant, the percentage of previously infected animals would still be as high as 2.4 per cent. (11 among 450 sera) titrated to the end point (Table II). In the following discussion all sera agglutinating to titre of 1:200 or higher are termed positive.

It will be seen from Table I that the percentage of sera with a positive agglutination reaction in a dilution of 1:200 varied in the different slaughter houses. In Jerusalem, 8.9 per cent. of the animals examined were found positive. In Tel Aviv the percentage of positive sera was 9 per cent. while the number of positive sera in the Kfar Saba district and in the Herzlia slaughterhouses was as high as 18 per cent. These differences are, presumably due to chance, as our numbers are small and the percentage of positively reacting animals varied from hatch to hatch, but it may be pointed out that not a single human case of leptospirosis was reported from the Jerusalem region, while the majority of human cases came from the Kfar Saba, Herzlia region.

Both locally raised cattle and imported animals are slaughtered in Palestine. We tried to determine the country of origin for the majority of the animals examined. The results are summarized in Table IV.

It will be noted that the highest percentage of positive sera was found among Palestinian cattle (11 per cent.). Among the cattle of Palestinian origin examined were 36 sera from water buffaloes ("Jarouch") five were positive in dilution of 1:200 (13.9

per cent). These animals live in swampy regions and the high percentage of positive sera obtained from them is interesting from an epidemiological point of view. *Leptospira* are water organisms and the buffaloes have certainly had ample opportunity to acquire their infection in water. Among cattle imported from Turkey the percentage of positive was 8.7 per cent, and among cattle imported from Iraq, 10 per cent. Two positives were also found among nine animals from Syria and the Lebanon. No claim is made that the percentages of "positive" animals represent the real frequency of the disease in the different countries. Our numbers are too small for such a conclusion. But it seems justifiable to conclude that infection with the bovine strain of *Leptospira* is present in a varying but rather high percentage among cattle from the countries of the entire Middle East.

TABLE IV
CATTLE ACCORDING TO COUNTRY OF ORIGIN

Origin of animals	Total number examined	Positive 1:200 or higher
Palestine	321	30 (9.2 per cent)
Turkey	240	21 (8.7)
Lebanon, Syria	9	2
Iraq	90	9 (10)
Unknown	209	15 (7.1)
Total	869	57 (6.5 per cent)

RELATION OF SERUM TITRE TO CARRIER STATE IN CATTLE

It would be of interest to determine the percentage of animals with a positive agglutination titre which are still carriers of *Leptospira*. In experiments reported earlier (BERNKOPF, OLITZKI and STUCZYŃSKI, 1947), the excretion of *Leptospira* in the urine was demonstrated 4 weeks after infection, *Leptospira* were demonstrated histologically in the kidneys of an infected animal 8 weeks after infection (UNGAR and BERNKOPF, 1947). In order to learn something about the upper time limit for the excretion of *Leptospira*, the following examination was undertaken.

In the course of one of our series of agglutination tests, pieces of kidney were taken from slaughtered cattle together with the blood, and the tissue pieces were fixed in formalin. Tissue samples of five kidneys from animals which had been shown to have agglutination titres of 1:200 or higher were stained by Levaditi's method and were examined for *Leptospira*. No organisms were found. Similar negative results were obtained when we attempted to demonstrate *Leptospira* in the urine of cows from the settlement of Tel-Amal, which had passed through an epidemic of leptospirosis one and a half years previously. Among 65 cows examined serologically, 54 animals were found to be positive. Urine was taken from 20 of these animals and was spun rapidly in an angle centrifuge. The sediment was examined for *Leptospira* with the aid of the dark-field microscope. No organisms were found. It is therefore supposed that only a small percentage of serologically positive animals, presumably those which have only recently been infected, are still *Leptospira* carriers.

EXAMINATION OF SERA OF RATS.

Rats are known to be carriers of *L. icterohaemorrhagiae* and other strains of *Leptospira*. In order to decide if they also carry the bovine strain of *Leptospira*, the sera of 509 rats were examined serologically. The rats were caught at the following places

Two hundred and twenty rats from the neighbourhood of Jaffa and Tel-Aviv caught for the Government Laboratory for plague examination, Jaffa. We examined these animals with the kind permission of Dr. AJOUX the M.O. in charge of this laboratory

Thirteen from the premises of the Tel Aviv slaughterhouse.

Eighteen from private houses and farms where cases of leptospirosis had occurred a short time previously

Two hundred and fifty-eight from Ramallah-Herzlia and the neighbourhood, which is situated in a region where human cases occurred, and where a high percentage of slaughtered cattle were found to be positive.

Most of the rats belonged to the sub-species *Rattus rattus alexandrinus* and *R. rattus*, and a few of them belonged to the species *R. norvegicus*. The mouth-to-tail length of all the animals was measured and 110 of them were 20 cm. long or more. The sera were tested against the bovine strain of *Leptospira* and against *L. icterohaemorrhagiae* in dilutions of 1:20 and 1:200. All the sera were completely negative against the bovine strain. It is concluded from these findings that rats are not carriers of the bovine strain of *Leptospira*. Two rat sera from a farm in Ramallah reacted in titres of 1:20 and 1:40 against *L. icterohaemorrhagiae*. The sera from 20 additional rats and of two cats all from this farm were examined and found negative. The positive result with *L. icterohaemorrhagiae* seems inconclusive, because of the low titre of the sera. It must be pointed out, however that three infections with *L. icterohaemorrhagiae* occurred in the coastal plain during the past year: two of them in the neighbourhood of the site where the two animals with positive sera had been found.

EXAMINATION OF SERA FROM PEOPLE WORKING IN SLAUGHTERHOUSES.

Since people working in slaughterhouses are particularly likely to be exposed to infections with the bovine strain of *Leptospira*, the blood of 207 such persons from the slaughterhouses of Jerusalem, Tel-Aviv, Herzlia and Kfar Saba was examined. Four positive sera (2 per cent.) were found among them (two of them from the Kfar-Saba and the Herzlia slaughterhouses). Only one of them reported a previous attack of leptospirosis in which the disease had been recognized serologically and clinically: two others reported an undiagnosed severe disease in the past (one typhoid like, and one nephritis); the last could not recall having had any disease since he took up this profession. ALSTON and BROWN (1935) reported that among 45 sewer workers nine (20 per cent.) gave positive serum reactions with *L. icterohaemorrhagiae*. This

percentage is much higher than that found by us (2 per cent) One possible explanation for the difference may be found in the fact that infected rats, the source of infection of the sewer workers, excrete *Leptospira* during their whole life while the infectivity of bovines, the source of infection of workers in slaughterhouses, seems to be limited to a short period during and after their infection

SUMMARY

1 The sera of 869 head of slaughtered cattle were examined for agglutinins against the bovine strain of *Leptospira* Eighty-three sera (9.5 per cent) gave a positive reaction in a dilution of 1:200 or higher

2 Five hundred and nine rats were examined for agglutinins against the bovine strain of *L. icterohaemorrhagiae* None was positive against the bovine strain of *Leptospira* and two reacted in dilutions of 1:20 and 1:40 against *L. icterohaemorrhagiae*

3 Two hundred and seven workers in slaughterhouses were examined for agglutinins against leptospires, and four positive sera were found

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THERAPEUTIC EXTRADURAL BLOCK IN TROPICAL ULCER

by

O. N. RANSFORD, M.D. (LOND.) D.A.M.C.*

In his "Researches in Medicine and Other Addresses," Sir THOMAS LEWIS draws attention to those diverse, aching examples of a human need for palliation invite research and promote knowledge which is to become useful to the health of humanity. In tropical ulcer we have an outstanding example of such a need. It is a disease process which every year causes an infinite amount of suffering among the undernourished inhabitants of the tropics. It is a condition which has been investigated by many of the practitioners who have come into contact with it, but the very varied views that are held regarding its aetiology testify to the lack of true knowledge that we possess about its cause. The large numbers of papers which are every year published on the subject of treatment are eloquent of the therapeutic difficulties encountered.

This paper is based on observations made upon a large number of cases of tropical ulcer seen in East Africa and Nyasaland. Like many other workers, the writer has come to regard the condition as an infection imposed upon a localized area of skin ischaemia, and has been dissatisfied with the results of treatment directed solely against the infective part of the syndrome.

* I am indebted to the D.M.S., Nyasaland, for permission to publish this paper.

The Site of Election of Tropical Ulcer

SAINT (1945) has pointed out that there are marked priorities in the distribution of blood to the tissues, and that the skin of the lower limb is of the least importance. That the lower part of the front of the legs and ankles has an unusual blood supply has been recognized by many workers, and to this abnormality has been attributed the high incidence in this area of the lesion of tropical ulcer (CONNELL and BUCHANAN 1933). Here the blood supply to the skin is maintained through terminal blood vessels of adjoining arteries which fail to overlap. WOOLARD and WEDDELL (1935) have pointed out that these same vessels are unusually richly supplied with vasoconstrictor fibres.

TROPICAL ULCER IN NYASALAND.

Like their neighbours in East and Central Africa, a large proportion of the Africans in Nyasaland live on a suboptimal diet—they are, furthermore, a prey to heavy helminth infestation. The impression that the observer receives is that like the condition of prisoners of war in the Far East, the natives become stabilised at a low level of health. Only rarely do they show the classical florid syndromes of deficiency states. In their condition adverse factors such as some specific disease or prolonged activity aggravates the existing deficiency and brings it to light.

Of the signs of deficiency in the peasant in Nyasaland, follicular prominence over the thighs and buttocks, due perhaps to stimulation of the *erectores pilorum* muscles, and a hairless dry atrophic condition of the skin over the shins are very frequently seen. PLATT (1947) has recently recorded data of the signs of nutritional health in Nyasaland. Among them he observed that the skin was atrophic in 63 per cent. of the inhabitants of three villages, that xerosis was found in 74 per cent., and a condition of "permanent goose flesh" in 43 per cent.

A long description of the appearance of tropical ulcer is not justified in view of the extensive literature already in existence. In his review CONNELL (1936) notes many relevant features which the ulcers seen in the Sudan share with those found in Nyasaland. Five points regarding their occurrence in the latter territory may be considered to be worthy of emphasis.

(1) The incidence is seasonal, and is highest towards the end of rains. This is reflected in the heavy hospital attendances some 4 to 8 weeks later. This high incidence coincides with seasonal food difficulties, and the hardest work in the fields. A similar relationship was seen in recruits for the East African forces in whom high incidence in 1942-43 coincided with the prevailing food scarcity among the civilians.

(2) A large proportion of ulcers develop spontaneously from a preceding small blister and there is in these cases no history of trauma. I series of 322 cases seen in the central province of Nyasaland, 147 began as idiopathic cracks, while in the Southern Province 164 out of 319 began in this way.

These figures approximate to CONNELL's 4 per cent. The impression gained was that the ulcers of spontaneous origin run a more malignant course than those which followed trauma.

(3) An associated condition of marginal gingivitis was prevalent from which the *fusio-spirochaetes* so commonly found on the surface of tropical ulcer were recovered.

This represents a reservoir of organisms for transfer to the blister and the abrasion which follows it. The African is an inveterate nail biter, and in addition often relieves with spittle the itching caused by the blister.

(d) Ulcers on the toes never begin with a blister. Most are the result of jigger infection.

(e) Clubbing of the fingers has been observed in a significant proportion of tropical ulcer cases. In this connection it is of interest to note that BROCKENBANK (1913) has observed X-ray changes in West Africans suffering from tropical ulcer which resemble hypertrophic osteoarthropathy, of which clubbing is a manifestation and for which tissue anoxia is held to be responsible.

A THEORY OF AETIOLOGY OF TROPICAL ULCER

There can remain little doubt that an essential predisposing cause of tropical ulcer is malnutrition. In this paper it is postulated that the occurrence of the ulcer is preceded by and conditioned by a local skin rechemism, itself due to vasospasm. It is profitable to consider the deficiency states in which changes in the autonomic nervous system and in the peripheral blood vessels have been described.

In beriberi deficiency of the vitamin B complex is known to affect the central nervous system. There is evidence that the autonomic nervous system likewise may be affected. Thus VERNON (1935) describes degenerative changes in the sympathetic nerves in beriberi, and in 12 cases of the same condition coming to autopsy. BLAIR ARNOLD *et al.* (1946) have observed swelling and chromatolysis in the cells of the autonomic system.

In pellagra too similar changes have been recorded. LANGWORTH (1931) deals fully with the autopsy findings in a case where accumulation of fat and pigment deposits were found in the cells of the autonomic ganglia while some cells showed chromatolysis and nuclear displacement. Similar changes in the sympathetic ganglia of pellagra cases have been remarked by other workers (SMITH & STITT 1945).

The effects of such nervous changes are reflected in the peripheral blood vessels. PAGE (1946) described cases of paresthesia of the feet associated with beriberi and pellagra. He notes that KOTOSHITA after study of the same condition in Japanese troops returning from the South Pacific, indicated that the crucial changes were primarily found in the vascular system which showed narrowing or obliteration of the lumen of the small arterioles of the feet and legs. More recently GILLMAN (1947) has suggested that a condition of bilateral gangrene of the feet seen in a Mashona native was due to a nutritional defect in vessel walls which followed deficiency of one or more factors of the vitamin B complex.

The syndrome of 'burning feet,' occasionally seen in Nyasaland natives, has elsewhere been the subject of a number of reports, and is attributed by some workers to a condition of local vasospasm. Thus MORGAN, describing the condition in Tamil labourers in 1929, ascribed it to a deficiency of vitamin B₁ group which caused an over-stimulation of the sympathetic system. He derived benefit from therapy with the vasodilating drug nitroglycerine. Similar improvement in the condition has been reported more recently by HARRISON (1946) using intravenous calcium, nicotinic acid and amyl nitrite. Several writers have obtained successful results following the exhibition of vitamin B₁ alone.

There is some evidence that in cases of tropical ulcer there are signs of a vitamin B deficiency state. Thus CROSS (1900) found a degree of anaesthesia in the ulcer area, in the first cases recorded in Nyasaland, and GILLMAN (1941) observed that in Naga sores there were, almost invariably, at least minimal signs of beriberi.

THERAPEUTIC SYMPATHETOMY

Turning to the effects of arterial occlusion on the legs, it results in the objective signs of atrophy of the calf muscles, and nutritional skin disturbance

as manifested by atrophy and deformity of the nails, a shiny skin, disturbed hair growth, loss of perspiration and objective coolness. These are all signs which are found in a ulcer prone community in Nyasaland.

In health the blood flow through the skin is extremely labile in order that its function of a radiator surface may serve. When the blood flow falls below a level consistent with a proper nutrition of the skin a definite march from a preliminary wheal or blister to necrosis occurs. This progression is seen in the development of a bedsore and is mirrored in the stages of the spontaneous type of tropical ulcer and furnishes additional evidence that this latter lesion is primarily due to local vascular occlusion—that it is, in fact, a local gangrene.

Attention has recently been focused upon conditions of impaired circulation and nutrition in which the blood vessels are at fault. The operation of sympathectomy is now frequently performed in a number of pathological conditions with the intention of restoring the blood supply to normal by removal of sympathetic activity. The list of these conditions includes erythromyalgia, indolent ulcers, Volkmann's contracture, and thrombo-angiitis obliterans. In experimental animals, JOHNSON *et al* (1932) showed that after sympathectomy the vessels regain a degree of independent tonus. In man there is a wealth of evidence to show that though a certain amount of tonus does return, it does not reach the abnormal preoperative level. EDWARDS (1947) has suggested that sympathectomy causes benefit by removing the variability of the skin circulation.

The sympathetic innervation of the ulcer-prone part of the leg requires consideration. It is supplied by a caudally directed stream of sympathetic fibres arising from the connective cells of the lateral horn of grey matter lying between the 10th thoracic and the 2nd lumbar segments inclusive. The large majority of the fibres are derived from the lumbar segments. The vasoconstrictor fibres are then concentrated according to LUSCHKE (1899) in the first lumbar ganglion. After relaying in the sympathetic chain, grey rami communicantes enter the nerves supplying the lower extremity and are distributed to the arterioles, capillaries, veins and the recently recognized arteriovenous shunts. Of the vessel the arterioles receive the richest supply. Other fibres supply the sweat glands and arrectores pilorum muscles. The work of TOGO and KRAVETZ (1915) and of WOODWARD (1923) has shown that the supply to the vessels is not continuous short trunks in the muscular layer but that the plexus receives constant reinforcement from the parent trunk. Doubt has accordingly been thrown on the reported appearance of distal vasodilatation following perineural stripping, and LUSCHKE has ascribed his permanently good results derived from the operation to division of postulated afferent fibres which are concerned in reflexly determining vessel tone. This view has been vigorously opposed by LEWIS (1912).

ALTERNATIVE METHODS OF OBTAINING SYMPATHETIC RELEASE.

Clearly an operation of the magnitude of sympathectomy is not indicated in the routine treatment of tropical ulcers. Four other methods of inducing sympathetic release in the condition have been studied by the writer. Combined with local treatment of the ulcer they have been found to retard the phagadenic process and to promote healing. They suffer from the common disadvantage that in none can the sympathetic paralysis be regarded as permanent. Benefit results from a temporary increase in the blood supply to

the affected part, and from the re-establishment of a proper balance between the two halves of the autonomic nervous system, when the normal balance previously was upset by sympathetic preponderance

(a) *Periarterial Stripping*—Beneficial results in the treatment of indolent ulcers of the legs by periarterial stripping of the common femoral artery have been reported by a number of workers, including LAMBERT ROGERS (1931). More recently, KIRKALDY-WILLIS (1946) noted its value in a leprous ulcer of the foot. Theoretical objections have been raised to this method, to which reference has already been made, the present writer has abandoned its use in tropical ulcer, chiefly because of the African's dislike for operation

(b) *Lumbar Sympathetic Block*—This procedure requires the paravertebral infiltration of the lumbar sympathetic ganglia as they lie in psoas substance. In a personal communication, FARR (1943) described its good results in tropical ulcer, LAURE (1943) successfully treated the gangrene of typhus in this way, while SIMMONS (1945), has noted its value in the treatment of chilblains. The application of lumbar block for thrombosis of the lower extremities, first suggested by LERICHE (1934) has been well reviewed in *Anaesthesiology* (Editorial, 1943, 4, 3), and also by LESLIE WILLIAMS (1944). In phlegmasia alba dolens, impulses arising in the thrombosed vein are carried over the sympathetic system and produce spasm in the homolateral arterioles and venules. Prompt subsidence of all clinical manifestations are reported in a large series of cases after sympathetic block. GELFAND (1947, unpublished) states that he has used this method with very good results in idiopathic thrombo-phlebitis ("tropical phlebitis"). Lumbar block suffers from the disadvantages of a difficult technique and the necessary approximation of the needle to large vessels

(c) *Therapeutic Spinal Block*—Here advantage lies in the easy technique. Its successful use in the treatment of gastro-intestinal achalasia has been attributed by LANGTON HEWER (1944) to bringing the two halves of the autonomic nervous system once more into step. Of great importance are the good results obtained by CUBITT (1936) in the reflex anuria of incompatible blood transfusion, and by ROBERTSON (1946) in invoking sympathetic release in three anuric cases of Weil's disease. DOBBS (1947) has recently reviewed its application in other forms of anuria. PRICE (1945) has already recorded the beneficial use of spinal anaesthesia in the treatment of tropical ulcer. Drawbacks to the method, however, require consideration. There is the danger of a calamitous fall in blood pressure in a debilitated patient, a purely segmental sympathetic release cannot be obtained, while motor paralysis can hardly be avoided. There are also risks of post-spinal headache and meningitis. These are factors which the writer believes preclude spinal anaesthesia from being an ideal method.

(d) *Extradural Spinal Block*—As a means of obtaining sympathetic release, extradural block offers some advantages over the methods already noted

in the treatment of tropical ulcer. Although the technique is more difficult than spinal anaesthesia and the result less reliable, it is easier than periaxial stripping or block of the lumbar ganglia. It has the great advantages over spinal block that a segmental sensory and sympathetic paralysis is obtained without motor paralysis. Because there is no vasomotor paralysis above or below the selected segments, post induction fall in blood pressure is minimal. In tropical ulcer extradural block should normally be used only in those lesions which are unlikely to heal quickly with more conservative methods and when it is used, it should always be combined with direct medication to the ulcer. This method of obtaining sympathetic block opens up therapeutic possibilities in those conditions of anuria which have been benefited by spinal anaesthesia. Thus it is the method of choice in the anuria of blackwater fever in which a serious fall of blood pressure might have disastrous consequences.

The method, which has come into general use in South America, depends upon the introduction of anaesthetic solution into the epidural space. To DAWKINS is due much of the credit for introducing the technique into England. Caudal anaesthesia likewise depends upon epidural infiltration with anaesthetic solution, but the approach is different, and is less easy.

The epidural space is about 4 mm. in diameter and lies between the paretal and medullary layers of the dura. In the space there is a negative pressure especially noticeable when the spine is suddenly flexed. Anaesthetic solution introduced into the space diffuses upwards and downwards, and along the nerve roots in the intervertebral foramina, and it is here that the spinal nerves and sympathetic rami are anaesthetized. Because the two layers of the dura fuse at the foramen magnum it is not possible for anaesthetic solution to reach the vital centres in the medulla. Added advantages of the method are that the anaesthesia is almost entirely confined to sensory and sympathetic nerves, and that it can be induced anywhere in the cord. Thus it is used in thoracic surgery. The extent of anaesthesia depends upon the degree of vertical diffusion of the anaesthetic solution and is controlled by varying the amount used. A segmental anaesthesia is induced which does not affect the nerves lying above or below the point of introduction of the solution. For the clinician this represents an advance because temporary sympathetic release can be invoked on a regional basis. Further it can be repeated at intervals of a few days, and prolongation of its effect is obtained without prejudice to the patient.

TECHNIQUE

The technique used in the treatment of tropical ulcer followed that described by D. WICKS (1945). The patient lies on the affected side in a 10° head down tilt. A wheel of local anaesthetic is raised over the space between the 1st and 2nd lumbar vertebrae, and a spinal needle of narrow gauge introduced as for ordinary lumbar puncture but with the stylette omitted. To the needle is attached an Odor indicator which consists of glass capillary tube and which when placed in sterile water fills with the inclusion of small bubble. (A syringe can necker may be used in place of the indicator but is not so satisfactory.) When the point of the needle reaches the epidural space the negative pressure

causes the bubble to move a short distance towards the needle. In theory the patient's spine should be flexed from an extended position as the needle approaches the epidural space but my experience is that this movement of the patient is unnecessary. The indicator is then removed and 25 c.c. of nupercaine (1:1,500) is introduced slowly. A skin thermometer has been used by the writer to demonstrate the loss of sympathetic control that follows. Before operation it is strapped to the leg and left there until a constant temperature is recorded. After successful block has been obtained, a rise of temperature of 4° to 8° F is recorded.

Illustrative Cases—(I) Biliati, an African boy aged 11 years, suffered from an acute tropical ulcer. It had developed during the week from a blister of spontaneous origin. There was the usual pattern of atrophy of the skin over the front of the legs, follicular prominence over the front of the thighs, buttocks and extensor surfaces of the arms, and hyperkeratosis over the knee and finger joints. The ulcer, measuring 3 cm. in diameter was situated on the left leg, just above the external malleolus. Skin temperature at a point 3 inches above the internal malleolus was recorded as 90.6° F. Twenty-five c.c. of nupercaine were introduced into the epidural space at the level of the 1st lumbar space. A rise of temperature over the foot was noticeable to the hand almost at once, in 15 minutes it stood at 94° F. This ulcer, which was of the type likely to continue for some time to increase in size with conservative treatment, was healing well after 4 days and the boy was discharged cured 3 days later. No motor anaesthesia developed in this case. The ulcer was dressed throughout with saline compresses.

(II) Legina, an African girl aged 12 years, had for 3 weeks suffered from a tropical ulcer on the front of the lower part of the left leg, which began as a blister. The ulcer was oval in shape and measured 7 cm. long and 5 cm. across, smears showed fusiform bacilli and spirochaetes. The surrounding skin was unhealthy and had broken down above the large ulcer. Clinical experience suggested that despite ordinary medical treatment, the ulcerated area was likely to increase in size for some days. Temperature over the dorsum of the foot was noted as 89.8° F. Extradural block was induced as before, and the following temperatures were recorded—

3 p.m.	89.8° F	Extradural block
3 10 p.m.	94.2° F	
3 15 p.m.	95.2° F	
3 20 p.m.	96.6° F	
3 25 p.m.	97.2° F	

The condition of the ulcer improved noticeably over the next 4 days, during which a high leg temperature was maintained. At the end of that time the base of the ulcer was clean and healthy, surrounding skin appeared to be normal, and epithelial growth at the edges had begun. Further healing progressed uneventfully.

In cases of tropical ulcer, sympathetic block was followed by a rapid rise in temperature. This is an important point, suggesting that in this condition vascular obstruction is indeed spasmodic and its release abrupt. Where skin anoxia is due, on the other hand, to structural disease of the vessels, LEWIS (1946) has pointed out that any increase in skin temperature which may follow sympathetic block, appears slowly. Sympathetic block may be expected to improve the chances of healing in such cases, but its action cannot be regarded as specific because the lumen of the diseased vessels is probably incapable of much increase in size.

Illustrative Case—Kumachemba, an elderly male African, had suffered from two ulcers on the left leg for 3 years. The ulcers were oval in shape and adjacent to each other. They measured 7.5 cm. \times 3 cm. and 5 cm. \times 2.5 cm. respectively. They were situated on a badly scarred part of the leg, were clean, inoffensive and presented an appearance quite unlike that of tropical ulcer. Skin temperature of the foot was high (93° F) and only rose 0.2° over a period of half an hour following extradural block. I am indebted to Dr

W. O. PETTIT, under whose care this patient was, for reporting the subsequent progress of the ulcers. Within 7 days healing was practically complete and the man was discharged 11 days after block.

DISCUSSION

Lewis (1939) has drawn attention to the way in which the advance of medical knowledge has revealed that distinct diseases have superficial resemblances causing them to be confused on the other hand, conditions thought to be quite unconnected have proved to have a common pathological basis. As the art of anaesthesia has broadened its role to include therapy it has suggested that such diverse conditions as the reflex anuria of renal anoxia which has recently attracted so much attention (TRUETA, 1943; MARGRAITH, 1945) and Reynaud's disease have a relationship with tropical ulcer. In all, relief may be obtained by invoking sympathetic release of the vessels at fault.

In tropical ulcer it has been seen that the rapid increase of skin temperature which follows sympathetic paralysis suggests that the skin vessels of the lower limb are in a state of spasm. On other evidence this is the view held by COOK (1932) and others after consideration of the benefits obtained from calcium therapy. It is apparent, then, that in tropical ulcer there is a local condition of skin gangrene upon which fuso-spirochaetal infection is readily imposed, adding a second factor to what becomes a vicious circle. Treatment should accordingly be directed towards reversing the factor which causes ischaemia, and should deal with the infecting surface organisms as well. Many authorities have published evidence to show that tropical ulcer is a disease due to malnutrition. Evidence here reviewed suggests that the lesion is due to sympathetic hyperactivity induced by a condition of vitamin B complex deficiency. Some factor of nature must be presumed to account for the epidemic-like outbreaks which have been severally described. Such a factor may be a seasonal dietary change of a community which affects the synthesizing intestinal flora.

JAMES (1939) and PRICE (1945) have rightly held that vascular changes in the ulcer area places tropical ulcer outside the realms of ordinary medicine. Other communications have reported the promotion of healing which has followed sympathetic block. Here attention has been drawn to the advantages which extradural anaesthesia holds over other methods of invoking sympathetic release.

SUMMARY

Healing of the lesion of tropical ulcer can be promoted by the induction of regional sympathetic block over the affected area. The advantages of extradural block are compared with those possessed by three other techniques.

In cases of tropical ulcer the abrupt rise of skin temperature of the leg which follows sympathetic block suggests that the released vessels were in a state of vasospasm. It is postulated that sympathetic hyperactivity is responsible

for the vasospasm and affects the peculiar and vulnerable arterioles of the ulcer-prone lower leg. Evidence of morbid changes in the autonomic ganglia in vitamin B deficiency states is reviewed.

The aetiology of tropical ulcer may be summarized as "Skin anoxia and infection", in difficult cases treatment should be given for both factors

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A NOTE ON VITAMIN B COMPLEX DEFICIENCY STATES AMONG AFRICANS IN THE GOLD COAST

BY

J DAWSON,
G M FINDLAY

AND

R D WARD

Before the war considerable interest had been aroused by the occurrence of optic atrophy both in Sierra Leone and in Southern Nigeria, this atrophy being correctly regarded as due to a nutritional deficiency.

During and after the war investigations have shown that nutritional optic atrophy has a wide distribution. WILKINSON and AU KING (1944), in a nutritional survey of Hongkong, demonstrated that amblyopia was associated with other signs of deficiency such as acroparaesthesia, weakness of the extremities, palpitation, giddiness and oedema. One of the 14 patients with optic atrophy was a frank pellagrin. They therefore suggested that the amblyopia was caused by a nicotinic acid deficiency. SPILLANE and SCOTT (1945) reported a series of cases from the Middle East among prisoners of war, while many observers have noted optic atrophy associated with other deficiencies among prisoners released from internment under the Japanese.

Our own interest in nutritional amblyopia was aroused because of the occurrence of five cases of optic atrophy among African soldiers serving on the Gold Coast and living on the usual Army diet which had not previously given rise to any signs of optic atrophy among troops stationed in this area. Of the five patients with signs of atrophy, all exhibited gross contraction of the

fields of vision while one had signs of generalized polyneuritis. An interesting fact was that all the soldiers involved, although Gold Coast natives, had been stationed in Sierra Leone for from 1 to 3 years where, according to their statement, they had lived on a predominantly rice diet, in contrast to the more usual diet of cassava and yams as eaten in the coastal belt of the Gold Coast, or of millet, as eaten in the Northern Territories.

BIOCHEMICAL INVESTIGATIONS

In view of the polyneuritis associated with the optic atrophy in one case and the absence in all of skin and tongue lesions associated with deficiency of other factors belonging to the vitamin B complex, it was considered that the signs of optic atrophy might be manifestations of thiamin deficiency.

The biophosphite binding power of the urine was determined by the method of CLIFF and COOK (1932) while thiamin saturation test was also performed as described by HOCHBERG and MILARICK (1944). The saturation test was carried out using 5 mg. thiamin as the test dose. Observations on normal African subjects showed that the normal excretion in the urine over a 24-hour period after such test dose given by mouth was between 0.9 and 2.5 mg. The normal 24-hour excretion of biophosphite binding substances was equivalent to less than 50 ml. N thiosulphite. Values of over 100 ml. N thiosulphite were associated with marked drop in thiamin excretion in the thiamin saturation tests. In the five Africans with optic atrophy the biophosphite binding power of the urine was markedly raised. The biophosphite binding power of the 24-hour urinary output of these five Africans ranged between 120 and 200 ml. N thiosulphite while the excretion of thiamin in the urine over 24 hours' period after test dose never exceeded 0.5 mg. In view of these findings the cases were regarded as examples of thiamin deficiency. Treatment with 50 mg. thiamin daily for 7 days failed, however to bring about any change in the eye condition, although the polyneuritis in the one case was improved. Further treatment with riboflavin, nicotinic acid and multivite yeast tablets also failed, even though continued for 8 weeks, to improve the optic atrophy though the biophosphite binding power of the urine had returned to normal and on the basis of the saturation test the patients appeared to be fully saturated. Thus it appeared that the optic lesions, whatever their cause had progressed so far that they were no longer amenable to large doses of vitamins.

Further light, it was thought, might be shed on the problem whether thiamin deficiency was the primary cause of the optic atrophy by an examination of the remainder of the Gold Coast troops who had been stationed in Sierra Leone, since if early cases of optic atrophy could be detected their eye lesions might respond to thiamin therapy. As a comparison, an examination was also made of African troops who had served continuously for from 2 to 3 years in the Gold Coast, and of African civilians living on the diet usual in the coastal belt of the country. Four groups were thus distinguished:

- | | |
|---------|--|
| Group I | The detachment of troops which had returned from Sierra Leone |
| II | Troops which had never been out of the Gold Coast |
| III | Patients in civilian hospital. |
| IV | Civilians living in apparent health in the cocoa growing area of the Gold Coast. |

The investigations were as follows:

(1) *Clinical*—Examination for crary pavement skin, nasal seborrhoea, cheilitis, angular stomatitis, glossitis and absent knee jerks. These lesions are

all believed to be characteristic of a B complex deficiency. In addition, a fundal examination was performed and the acuity of vision estimated by means of Snellen test type.

(2) *Biochemical*—A sample of urine was obtained and the bisulphite binding power determined by the method of CLIFT and COOK (1932).

From the above investigations the following information was obtained

- (i) The extent of B complex deficiency in any group
- (ii) The probable existence of optic atrophy in any particular individual
- (iii) The probable existence on biochemical evidence of a thiamin deficiency

Persons who demonstrated a lesion of the optic discs compatible with optic atrophy and also persons showing a high bisulphite binding power had thiamin saturation tests performed by the method previously described. In addition, the fields of vision were determined using a perimeter.

The following were the results obtained by this method of survey.

Group I The African soldiers on returning from the Gold Coast had been split into two sub-groups.

The first sub-group, consisting of 90 men, lived on the normal adequate Army diet on their return. This consists of cassava and yams, a small amount of meat and ground nuts as the staple constituents. The diet was not in any way supplemented by produce grown by the Unit. Clinical examination revealed that eight had glossitis, two crazy pavement skin and scrotal eczema, and 14 had absent knee jerks. Many apparently healthy Africans have absent or very sluggish knee jerks. On biochemical examination, 13 had a raised urinary bisulphite binding power. Thiamin saturation tests were done on these 13 men, 11 were normal. It was also demonstrated that on a total 24 hours' output of urine the bisulphite binding power was normal. Two men were on the borderline of thiamin deficiency as their thiamin excretion after a test dose was 0.4 mg. These two men also had contracted fields of vision. Thus there were two cases of incipient optic atrophy with thiamin deficiency. Feeding these two patients with 50 mg thiamin daily for a week expanded their peripheral fields of vision so that vision became normal. It is also interesting to note that the bisulphite binding power carried out on isolated specimens of urine is not a perfect guide to the daily level at which thiamin is excreted. An attempt was made to allow for differing urine concentrations by taking the specific gravity of the urine and seeing to what approximate daily volume this would correspond. From this approximation it was possible to minimize the effect of varying concentration of urine on the bisulphite binding power.

The second sub-group was seen about 2 weeks after the first sub-group, by which time the men had been back in the Gold Coast for nearly 2 months. The diet was much superior to that of the first sub-group as the Army rations were supplemented by produce grown by the Unit. The men were able to have a ground nut soup every evening and vegetable soup in the middle of the day. On clinical examination, out of 47 men two had crazy pavement skin and one man had cheilosis and glossitis. One man had glossitis alone and one had scrotal eczema. On biochemical examination, 13 showed a raised urinary bisulphite binding power. Thiamin saturation tests, however, showed that none of these cases was thiamin deficient. Perimetry revealed no abnormalities. This group appeared clinically to be much healthier than the first, and this would appear to be borne out by biochemical observation.

Group II Thirty-eight African soldiers who had never left the Gold Coast, and who had been stationed in the same Unit for 2 to 3 years, were also examined. None showed any clinical signs of B complex deficiency. Biochemically, six had raised urinary bisulphite binding power, but none was deficient when tested by the thiamin saturation test. No optic changes were seen.

Group III Hospital patients in civilian hospital were examined: they had not been admitted for nutritional deficiencies but were accident cases or cases of chronic infection. Thirty-one male patients were examined. 14 had skin and tongue lesions characteristic of B complex deficiency. Crazy pavement skin was extremely common. One of the patients, suffering from tuberculosis had in addition frank pellagra. Fourteen men showed raised urinary bisulphite binding power, four of these showing no clinical evidence of B complex deficiency. No evidence of optic atrophy was found. This series illustrates how near the African appears to be to serious nutritional disorders. An accident or infection may be sufficient to transform an incipient deficiency state into manifest disorder.

Group IV Of 80 civilians from the lower economic levels, all with active yaws 49 had B complex deficiencies indicative chiefly of riboflavin deficiency. Crazy pavement skin was again very common. 9 per cent. had raised urinary bisulphite binding power. A further 72 persons from higher economic level were also examined. 4 showed lesions characteristic of B complex deficiency chiefly lack of riboflavin. Crazy pavement skin was again very common. 8 per cent. had raised urinary bisulphite binding power. No evidence of optic atrophy was found.

From this investigation, the following general conclusions can be drawn.

(1) The normal Army diet appeared to afford a greater degree of protection against deficiency states than did the civilian diet. As the Army diet was the produce of the Gold Coast, it is possible to devise, from local materials, a diet which would be adequate.

(2) Africans normally live on the borderline of deficiency states. Hospital patients demonstrate quite clearly how deficient the diet must be in factors of the B complex.

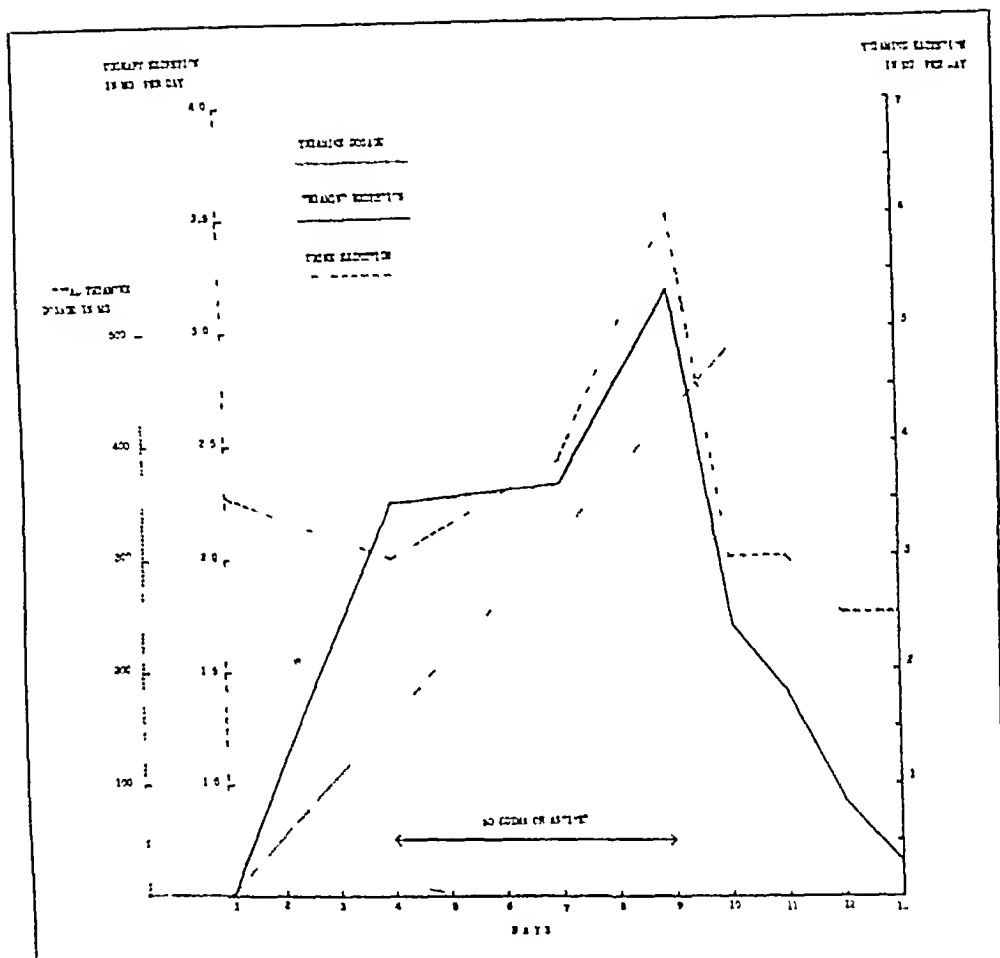
The following case illustrates the diversity of pathology which may occur in association with thiamin deficiency.

An African soldier was seen with gross ascites, oedema of feet and ankles and slight jaundice. On examination the patient's liver was enlarged, distance of two finger breadths below the costal margin, the outline being irregular. No optic atrophy was found, the albumin/globulin ratio was 0.3. A diagnosis of cirrhosis of the liver was made. On the basis of the thiamin saturation test the patient was found to be grossly deficient.

Treatment with thiamin was initiated and the Graph illustrates the relation between output of thiamin in the urine, urine volume and total thiamin intake.

Between the fourth and ninth days the urine output increased markedly and as the patient approached thiamin saturation the oedema and ascites disappeared. During this period the albumin/globulin ratio returned to normal. At this point thiamin dosage was stopped. Urinary output decreased markedly and thiamin output in the urine diminished almost to nil. Within fortnight the oedema and ascites had reappeared and the patient was again thiamin deficient. The albumin/globulin ratio was again reversed. Further thiamin treatment improved the condition. The patient was then discharged from the Army on medical grounds and could not be followed further.

The interesting features of this case are that the liver condition appeared to interfere with the normal retention of thiamin. In addition, the alterations seen in the albumin/globulin ratio would appear to indicate that thiamin exerts some influence on the formation of albumin. As the total plasma protein throughout the experiment was within normal limits, the decrease in the albumin concentration was probably responsible for the appearance of the oedema.



DISCUSSION

Evidence is brought forward to show that the dietary deficiencies to be met with in the Gold Coast and Sierra Leone are not absolutely identical. In the later colony the use of rice as a primary foodstuff increases the thiamin deficiency with which the tendency to optic atrophy is linked. In early cases of optic atrophy thiamin produced improvement but in advanced cases thiamin, together with other B vitamins, had no action. In the Gold Coast no evidence of optic atrophy was detected but in patients with chronic diseases thiamin deficiency may be revealed biochemically. The diet on which the bulk of the population of the coastal belt of the Gold Coast live, would appear to be deficient not only in protein but in many factors of the vitamin B complex. These

multiple deficiencies were rectified by the Army diet given to African soldiers despite the fact that the diet consisted of foodstuffs grown in West Africa.

CONCLUSIONS.

Optic atrophy occurred in African soldiers who substituted rice for millet, yams and cassava in their diet. In the earlier stages it was curable by large doses of thiamin.

Optic atrophy was not found among African civilians living in the coastal belt of the Gold Coast, although their diet reveals many deficiencies which, as shown by the African Army diet, can be rectified.

Tests for the bisulphite-binding power of the urine should be performed on the total output for 24 hours.

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THE DIAGNOSIS OF SCHISTOSOMIASIS IN SOUTHERN RHODESIA BY THE RECTAL BIOPSY TECHNIQUE

BY

MICHAEL GELFAND, MB, MRCP, DMR,*

Government Medical Officer, Salisbury Native Hospital, Salisbury, Southern Rhodesia

The diagnosis of intestinal schistosomiasis is not simple. Many cases of the disease fail to be recognized by the various methods employed for its diagnosis. Microscopical examination of the stools does not always yield positive results, even though the subject harbours the parasite. I have seen patients in whom *Schistosoma mansoni* was not found in the excreta in the living subject, yet at autopsy, on digesting the rectum, ova of *S. mansoni* were found. A number of cases are thus inevitably overlooked during life. As a result, a search for a more positive method for diagnosing intestinal bilharziasis has been made.

Methods, depending on the humoral responses of the body, for the diagnosis of bilharziasis, such as the intradermal skin test, may be of value in certain cases, but they fail to prove the definite presence of the parasite in the host. A positive skin test, for instance, while suggestive, is not definite proof that the patient is infected, as false positives do occur. Further, a negative reaction does not mean that the patient is not suffering from the disease. F. HERNANDEZ-MORALES and JOSÉ F. MALDONADO (1946), using the cercarial antigen skin tests, obtained a certain percentage of negative results in patients passing ova. Sixteen cases were tested, of which three gave negative results and two were doubtful. As the authors point out, the most important drawback to the skin test is the personal factor in the interpretation of the results. The skin test is difficult to interpret. The criteria of a positive result, particularly as to the size of the weal, as pointed out by FAIRLEY and WILLIAMS (1927), should be adopted. If this were done, there would be less difficulty in the interpretation of positive results.

For some time it was known that ova of *S. mansoni* are more likely to be found in the periphery, and more particularly in the mucoid collection around the faecal mass, than in the central portion of the stool. Ova are shed into the lumen and would naturally be collected by the faecal mass at its periphery (GIRGES, 1929). This led KHALIL and SALEH EL DIN (1930), to introduce the rectal swab method. A specimen is procured by gently scraping the mucous membrane of the rectum with the rubber glove or rubber finger lubricated with soap. When the finger is withdrawn it is usually covered with mucus and often with a little faecal matter. These are spread on a glass slide and teased with a drop of water. KHALIL and SALEH EL DIN reported that this method gave

* I have to thank Dr R. M. MORRIS, Medical Director of Southern Rhodesia, for his kind permission to undertake this work and to publish this paper.

more positive results than any other. They found that in a routine examination of 58 cases, 70 per cent. were shown by the microscopical examination of the stools to be harbouring the disease, whereas by the rectal swab technique 83·1 per cent. of the cases gave positive results.

Perhaps, as a natural sequel to the rectal swab method, a very important development in the diagnosis of intestinal bilharziasis was made by OTTOLINA and ATENCIO (1943). They found, at autopsy, that the rectal mucosa is the site of the greatest concentration of *S. mansoni* ova. They confirmed these results by procuring small pieces of rectal mucosa in living patients through a proctoscope. By means of a biopsy forceps, a tiny piece of rectal mucosa was removed from the right dorso-ventral rectal fold, situated about 8 cm. from the anus. The sample thus removed was then digested in 5 c.c. of a 4 per cent. solution of potassium hydroxide and incubated for 3 to 4 hours at a temperature of 60 to 80° C. After centrifugation, the sediment was examined for ova. OTTOLINA and ATENCIO showed that the biopsies on 12 patients passing ova were all positive. Further, in 100 unselected patients, whose faecal examinations were negative, they found the biopsies of 11 revealed ova of *S. mansoni*. They suggest that multiple or successive biopsies of different parts of the rectum would increase the figures still more.

This important work was followed up by two workers of the Puerto Rico School of Tropical Medicine—HERNANDEZ MORALES and JOSÉ F. MALDONADO. They confirm, in the main, the work on the biopsy technique. In their series of cases a single biopsy was performed on each patient and a second only when deemed necessary. They compared their results with those obtained by special microscopical concentration techniques employed on faecal samples. They selected the first rectal valve for their biopsies. The tissue removed was pressed between two glass slides and examined immediately by a low power. Thus live eggs could be distinguished from dead ones. Altogether 138 cases were investigated. In the untreated group they found the presence of eggs in 100 per cent. by the biopsy method, whereas only 41 per cent. showed positive results by the microscopical concentration method. Forty per cent. of the patients had live eggs against 60 per cent. with dead ones. In the treated group the figures revealed that in spite of treatment the rectal biopsy was positive in 70 per cent. of the cases, but the microscopical examinations of the stools were positive in only 18 per cent. They thus showed the value of the rectal biopsy technique not only in the recognition of the disease but also in determining the efficacy of cure.

Approximately 8 months ago I decided to try this procedure on male natives admitted to the Salisbury Native Hospital. The cases selected were young adults, and they were divided into three classes:

- (1) Those passing ova of *S. mansoni* in the stools, but no ova of *S. haematobium* in the urine.
- (2) Those passing ova of *S. haematobium* in the urine, but no *S. mansoni* in the stools.

(3) Those passing no ova in the urine or stool, as determined by microscopical examination

As a rule, only one specimen of stool and urine were examined. The cases selected were prepared as for sigmoidoscopy. An hour before, each patient was given a saline enema of 1 pint. This is important, as without it the rectum was often filled with solid faecal matter, rendering the examination difficult. No sedative was given to any of the patients, but each was carefully instructed as to what was going to be done and what was required of him. After the sigmoidoscope was inserted, the first right dorso-ventral valve was located and a snip taken from it. The instrument was then passed to the second valve of Houston, and another snip taken. Then one or two more snips were taken from between these two regions, generally from a haemorrhage focus or other suspicious-looking area. The snippings were tiny, about 2 mm in size. The area bled, but only slightly, and as a rule the patient felt a sense of pain when the snip was taken. These snippings were placed on a clean glass slide, teased with a little water, covered with a coverslip and examined with a low power.

Results

1 Of the 38 patients passing ova of *S. mansoni*, 32 (84 per cent) showed ova. Of these, 17 (45 per cent) revealed viable ova of *S. mansoni*, seven (19 per cent) ova of *S. haematobium* (five viable and two non-viable), and eight (21 per cent) ova of both *S. mansoni* and *S. haematobium*.

2 Of 15 cases passing ova of *S. haematobium* in the urine, but no *S. mansoni* in the stools, seven (46·7 per cent) showed positive snippings. Of these, all seven had ova of *S. haematobium*, but two (13·3 per cent), in addition, had *S. mansoni*. Of the seven with *S. haematobium*, the ova in four were non-viable, and of the two with *S. mansoni*, one was non-viable.

3 In a series of 18 cases in which no ova were found by microscopical examination of stools and urine, seven (38·7 per cent) showed ova in the biopsy snippings. Of these, five (27·8 per cent) contained ova of *S. haematobium* (four were non-viable), and two contained ova of *S. mansoni* (both viable).

DISCUSSION

This procedure of biopsy snippings is one which will assist in the recognition of schistosomiasis. Whilst my results with regard to *S. mansoni* in the rectum did not yield such a high percentage of positive results as those of other workers, yet they do show that not only are ova found in the majority of cases passing ova of *S. mansoni*, but also that ova are found in cases passing neither ova of *S. mansoni* in the stools, nor of *S. haematobium* in the urine. The method may thus be used for cases suspected of bilharziasis with constantly negative urine and stools. My results show that stool examination is still important and cannot be discarded, since patients passing ova in the stool may still be negative with the biopsy technique.

My work has also revealed an interesting fact that ova of *S. haematobium*

(either viable or non viable) may be found in rectal snippings. Such ova may be found together with *S. mansoni* or alone. For instance, in a case known to be passing ova of *S. mansoni* in the stools, but with none of *S. haematobium* in the urine, ova of *S. haematobium* may be found in rectal snippings. Further a case not passing ova in the specimens of urine and stools examined may yield *S. haematobium* in the snippings. This result is not altogether surprising as ova of *S. haematobium* are often deposited in the tributaries of the inferior haemorrhoidal vein. It is believed that the adults of *S. haematobium* make their way to the vesical venous plexuses from the liver down the portal vein via the inferior haemorrhoidal vein. Therefore ova may be deposited in the rectal wall. Whilst the ova of *S. haematobium* for some unknown reason, do not always appear in the stools, yet FAIRLEY (1919), refers to this not infrequent event in Egypt. Similarly LOVETT-CAMPBELL and ROSE (1936) mention that not infrequently their cases in West Africa pass ova of *S. haematobium* in the stools. In my experience this is uncommon (under 3 per cent.). On the other hand, in a series of 102 cases proved at autopsy to have urinary bilharzias of the bladder on digesting the rectum of each in caustic potash, I found ova of *S. haematobium* in 77. It is not surprising, therefore, that ova of *S. haematobium* will often be found in the rectal snippings of a patient suffering from urinary bilharzias.

SUMMARY

1 The rectal biopsy technique was employed in the diagnosis of schistosomiasis.

2 Of 38 patients passing ova of *S. mansoni* in the stool but with no ova of *S. haematobium* in the urine, 32 showed ova in the rectal snippings. Of these, 17 showed ova of *S. mansoni* alone, eight of *S. mansoni* and *S. haematobium* together (double infection), and seven of *S. haematobium* alone.

3 Of 15 patients passing ova of *S. haematobium* in the urine but no *S. mansoni* in the stool, seven showed ova in the snippings. All seven showed ova of *S. haematobium* whilst two of them contained ova of *S. mansoni* as well.

4 Of 18 patients in whom ova were found in neither the urine nor the stool, seven showed ova in the snippings, and of these, five showed ova of *S. haematobium* and two of *S. mansoni*.

5 This method is of value not only in the recognition of *S. mansoni* but, in areas such as Rhodesia where *S. haematobium* is also endemic it has an added advantage.

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LYMPHOSTATIC VERRUCOSIS IN THE FORT HALL DISTRICT OF KENYA

BY

MALCOLM CLARK, MRCS, LRCP, DTM & H,*
Medical Officer, Fort Hall

LOWENTHAL (1934), working in Uganda, described a verrucose condition associated with chronic oedema to which he gave the name lymphostatic verrucosis. Lymphostatic verrucosis, as LOWENTHAL pointed out, is frequently confused with the condition known as mossy foot, to which it has a superficial resemblance, but it is in reality a separate clinical entity.

Lymphostatic verrucosis is very common amongst the African tribes living round the foot of Mount Kenya and Aberdare Mountain Range. The writer, working in Fort Hall Hospital on the lower foot-hills of the Aberdare Range, has seen over 200 cases of this condition in varying stages of severity in the last 3 years. In the Fort Hall Native Civil Hospital there have been as many as a dozen cases under treatment as in-patients at one time, and it is rare not to have one such in the wards. Milder forms are frequently seen in the out-patient department, usually in patients attending for some other complaint.

The clinical features are

(1) Oedema of the leg, sometimes spreading as far as the knee but never above the knee, usually bilateral but sometimes unilateral even in the severest cases.

* My thanks are due to Dr TIMMS, of the Medical Research Laboratory, Nairobi, for all the work he has done both in examining sections and in attempting to isolate the causal organism, also to Dr SEQUIRA, of Nairobi, who has seen some of my cases and has given me much kind advice, and to Dr McCLENNAN, Director of Medical Services, Kenya, for permission to publish this paper.

(2) A barnacle like growth of the foot associated with oedema, but unlike the latter *never spreading much above the ankle joint*. No other part of the body is ever affected.

(3) Ulceration—a late manifestation—which usually starts at the toes and may progress to such an extent as to necessitate amputation.

(1) The most striking feature of the oedema is its constant limitation to the leg below the knee. Even in the most advanced cases I have never seen the oedema extend above the knee. Lowenthal found that in three of his 11 cases the oedema was secondary to some other condition, but this has not been *my experience*. In no case that I can remember has the verrucous of the skin been associated with oedema of diseases such as chronic nephritis or lymphatic obstruction from some known cause. I have come to regard this oedema as the essential feature of a clinical entity and not as secondary to some other condition. The oedema pits readily in the early stages when it is best seen on the dorsum of the foot. But in cases of several years standing the swelling becomes hard and fibrous and the whole leg below the knee may be truly elephantoid, in the literal meaning of the word. The whole leg is much enlarged, the outline of the foot is lost and the skin is thrown into thick folds especially in front of the ankle. The patient walks with difficulty lifting each leg with care and with obvious effort.

(2) The barnacle-like or verrucose condition of the skin is the characteristic feature. In appearance it is a warty out-growth like thick dry moss, or more nearly like the incrustations of barnacles seen on wood long immersed in the sea. The edge of the growth is well demarcated and this is most noticeable. The growth is not painful on pressure nor does it bleed readily on slight injury. It occurs most commonly on the outer side of the foot, extending about one and a half inches above the sole. It is often found over the tendo achillis when it spreads upwards for some two and a half inches and on the inner side of the foot, but not on the sole except in very severe cases, and then only underneath the arch of the foot. It never develops on the parts of the sole which are subject to pressure. Scattered patches are common on the upper surface of the toes and in this situation are usually the first to ulcerate. The tips of the nails are often raised by the growths. Rarely is the whole of the dorsum of the foot involved, but scattered patches may appear here especially in front of the ankle, and the growth seems never to extend more than an inch at most above the joint. Round protruding fibromata, often an inch in diameter may be present in the late stages chiefly near the toes or more rarely near the heel. In very advanced cases of many years standing when the leg is elephantoid, the verru-
cous appear to atrophy leaving depigmented areas.

3 Ulceration. This is probably only a secondary complication, but it may be a most serious one leading to marked disability and may be severe enough to necessitate amputation. The ulcers usually begin on the toes or in front of the ankle, and are indolent and very slow in healing.

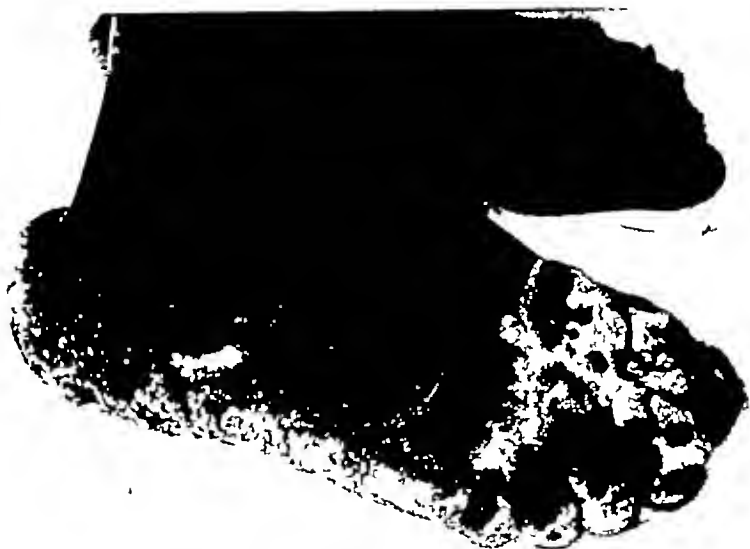


FIG 1 —The oedema can be clearly seen. The verrucose condition has its typical distribution—the sides of the foot, over the tendo achillis, in front of the ankle joint and on the upper surface of the toes. It does not spread above the ankle.

FIG 2 —Showing the oedema, verrucose condition and depigmented areas following atrophy.



FIG. 3 — In addition to the oedema and verrucous condition the round protruding fibromata are seen over the toes

FIG. 4 — Verrucous affecting the toes and sides of the foot. Depigmented areas left by the atrophy of the verrucous condition can also be seen

As has already been stated, the disease is generally bilateral, but many cases—even when far advanced—have been seen where the involvement is limited to one leg and in a careful examination of the other leg no trace of oedema, verrucosities, or ulceration can be discovered. When a careful history is taken in bilateral cases it is almost always found that the affection began in one leg some months or years before the other was involved. This is significant as it must raise the question of auto-inoculation from leg to leg, but no example of spread to the hands or any other part of the body has been encountered. The majority of the subjects are middle aged or elderly, some in the early teens have been met with, but small children and infants are never affected.

Both sexes are equally susceptible to attack. Several attempts to isolate a causal organism were made both by inoculation on to media and inoculation into rabbits, all these attempts met with complete failure.

As has already been stated, this condition is usually confused with mossy foot and it is by this name that it is still often known. THOMAS's original description of mossy foot, however, shows several fundamental differences from the condition here described. Mossy foot starts with a vesicular stage, it is painful and bleeds easily on slight injury, it attacks parts of the body other than the leg and, most important of all, it can be transmitted to rabbits.

Filarial elephantiasis is unknown in the Fort Hall area of Kenya. Repeated nocturnal examinations of blood during the last 3 years (including many cases of lymphostatic verrucosis) have never shown the presence of microfilaria and no case resembling classical elephantiasis of the legs, breast or scrotum has been seen.

Yaws is endemic in this area and has been stated to be the cause of verrucosis. But in each of 20 cases, three Khan tests were carried out with negative results in all. At one period all cases here were treated with novarsinobillon, but the treatment was without effect and has been abandoned.

This verrucose condition has been regarded (by LOWENTHAL amongst others) as merely a secondary and not very important development of elephantiasis. Even if this view were true, it does not explain why a form of elephantiasis should be limited to the leg below the knee, and usually—though not always—spread from one leg to the other leg but never to any other part of the body.

TREATMENT

As a routine measure, we now scrape all such cases, and I have observed some for over 2 years after treatment, and they appear to have derived permanent benefit. The growth does not tend to recur and the oedema and general thickening of the leg seem less. Ulceration does not recur in cases so treated. The growth is usually removed with a short amputation knife, it proves tough and fibrous and even in the most extensive cases bleeding is surprisingly slight.

CONCLUSIONS.

1 Lymphostatic verrucoma is a clinical entity first described and named by LOWENTHAL in Uganda in 1934

2 The condition is one of oedema limited to the leg below the knee, barnacle-like encrustations on the toes and foot but not progressing much above the ankle, and in late cases, ulceration.

3 It appears to be unilateral at the beginning but the second leg is usually affected later

4 The condition is very common in the Fort Hall district of Kenya and adjacent districts.

5 It is very slowly progressive over many years and may give rise to great disability even necessitating amputation.

6 All attempts to isolate a causal organism have failed

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BALANTIDIUM INFECTION ASSOCIATED WITH DIARRHOEA IN PRIMATES

BY

T A COCKBURN, M D, D P H *

An outbreak of diarrhoea occurred among the primates in the collection of the Zoological Society of London at Regent's Park in June to August, 1947. In the monkey house at this time were 45 primates, including some three drills, five baboons, three pigtail monkeys, and five chimpanzees. The chimpanzees are protected from droplet infection from the public and, indeed, are cut off from the main body of the house by large panes of glass.

In June, 1947, a pigtail monkey (*Macaca nemestrina*) was admitted to the sanatorium, having been off colour for some weeks and being much reduced in weight and strength. In particular, its gluteal muscles were much wasted and it had loose stools, some of them chocolate in colour and very fluid. Its face was very pallid, although its haemoglobin was 90 per cent, and red cell count 5,000,000. Examination of the faeces showed them to be heavily infected with *Balantidium*, the species of which has not yet been identified.

Two crab-eating monkeys (*Macaca irus*) in the same cage had their faeces repeatedly examined, but although the ciliates and cysts must have been swallowed by them on many occasions, the monkeys were never shown to be infected.

Treatment was initially commenced with the administration of mepacrine, gramme 0.1 daily, and this almost immediately cleared the pigtail's faeces of *Balantidium*. Unfortunately, a primate of this nature is very strong and difficult

* I am indebted to Dr REWELL, the Pathologist to the Society, Mr SKERTEN, who is in charge of the Sanatorium, and the keepers of the monkey house, for their assistance and co-operation.

to handle, and this one would only take the mepacrine mixed with large quantities of honey and so the treatment had to be altered. *Balantidium* reappeared in the faeces a few days later.

Carbarsone was next tried (JOURN and BURROWS 1934), and it proved a great success, for the pigtail considered the tablets a great delicacy and ate them without any difficulty. Within 24 hours of administration of grammae 0.26 b.d., all *Balantidium* had disappeared and the diarrhoea had improved. In 3 days the faeces appeared normal, and at the end of the 12 days' course of treatment the pigtail had largely recovered. After 3 weeks no *Balantidium* could be found in its faeces so it was returned to the monkey house.

In the meantime all 45 primates in the monkey house had been examined and eight found to be infected. Four of these had been known to have had persistent diarrhoea for some months, and these were given carbarsone for 12 days, with a dosage of grammae 0.26 daily for the small monkeys, and grammae 0.52 daily for the larger ones. In all cases the *Balantidium* and diarrhoea disappeared within 24 hours of commencement of treatment. The four symptomless carriers were left untreated.

Three weeks later a widespread epidemic of diarrhoea occurred in the monkey house and all primates concerned had *Balantidium* in their faeces. Those affected were three drills (*Mandillus leucophaeus*), three pigtails (*Maca nemestrina*), one *Cercopithecus torquatus lunulatus* and one *Cercoptithecus neglectus*. Some were those who had been symptomless carriers of the ciliates 3 weeks before and who had not been treated. One pigtail (not the one mentioned before) suffered severely and sat on a perch with liquid faeces running from him. In all cases carbarsone administration cured the diarrhoea within 24 hours, and on this occasion all primates with *Balantidium* with or without symptoms were treated.

The sudden onset of diarrhoea in so many primates, four of which had been symptomless carriers, is curious, and suggests that some other factor had come into operation. Perhaps the arrival of large numbers of cherries in the hands of visitors had something to do with it, although this by itself would not account for the outbreak. It would seem probable that four of the primates had acquired the infection during the preceding 3 weeks, for their stools had each been examined previously two or three times with negative results.

How the ciliates spread from one monkey to another is obscure. An effort is made by the keepers not to carry infection from cage to cage, and each cage has its own cleaning utensils. However, no primate can foul the wire of its cage and so its neighbour can pick up the infection. On the other hand, the cases in the monkey house occurred in all parts and not in adjoining cages, as might be expected. The spread of the cystic forms by air and dust is a possibility although they are said to die quickly when dried. Transmission by flies would seem more feasible, and this would explain the scattered distribution of the cases.

The brunt of the infection fell on the large primates and left the small ones almost untouched. Some of the monkeys were undoubtedly immune to infection, especially the crab-eaters (*Macaca irus*). None of the chimpanzees developed either diarrhoea or the infection with *Balantidium*, perhaps because of their isolation behind the glass partition.

Balantidia have been found in zoological gardens in many parts of the world in Baltimore (HEGNER, 1934), in Moscow (POPOW, 1928), in Berlin (CHRISTELLER, 1922), but for some reason there are practically no references to them in the Regent's Park collection. Professor J G THOMSON, in 1931-33, examined many animals and birds for protozoa, but found *Balantidium* only in an iguana. Colonel HAMERTON, the Society's pathologist, never mentions them in his reports, which cover a period of many years. MACKINNON and DIBB (1938) found a few cysts which might have been those of *Balantidium*.

The identification of the various species of the genus *Balantidium* is a matter of some difficulty. HEGNER (1934) lists 13 different species previously known and adds six new ones which he has discovered himself. No fewer than eight of these were obtained from primates.

There is very little mention in the literature of the epidemiology, symptoms and treatment of the infection in animals.

The discovery of *Balantidium* in an animal suffering from diarrhoea does not prove that the ciliate is the cause of the symptom. Primates often suffer from diarrhoea, for instance, if they are fed on certain fruits such as figs, prunes or cherries, loose and frequent stools will be forthcoming within a few hours, and if beetroot is given at the same time the stool will be stained deep purple or chocolate. Hookworms and other parasites can cause intestinal upsets, and tuberculosis in its final stages may have diarrhoea as a symptom. *Shigella flexneri* has caused epidemic dysentery in the monkey house, but this was easily distinguished by the sudden and widespread onset, with blood-stained faeces, high mortality and dramatic cure with sulphaguanidine powdered and mixed with milk. *Sh. sonnei* has also been connected with a small but serious outbreak of enteritis.

The cases under review were not caused by any of these agencies, and the circumstances were such as to suggest strongly that the symptoms of ill-health and enteritis of the primates were, in fact, caused by *Balantidium*.

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that the disintegration of DDT is accompanied by the emission of micro-waves, either electronic or sound, with which the antennae of *A. funestus* are in resonance, and which warn them of impending danger. Provided there is sufficient DDT on only a part of the habitation, placed in such a way that its reflection covers the whole of the region approached, the house should be safe.

An experiment has been started on these lines and at the time of writing encouraging results seem to be obtained. Five huts at Wobnar settlement lying just outside the area treated with DDT were chosen for the experiment. The habitations are along the edge of marsh about a mile long and are also surrounded by swampy patches here and there. The tenants are vegetable and rice growers. They live in huts made of thatch tied on framework of light poles, with roofs of cane trash or bulrush. Ceilings are absent, doors and windows are very few, some rooms have no windows, the door being the only opening. As these huts are low walled and have minimum of doors and windows, they are dark, maintain an even temperature, and are greatly favoured by anophelines. A survey of the settlement which has about 40 huts gave a total of 8,885 *A. funestus* and 177 *A. gambiae*.

In the experiment, huts 13 to 17 which produced the greatest numbers of anophelines, were chosen. Hut 13 had only one end wall and the roof above it sprayed. Hut 14 was kept as control. In hut 15 one-half of one long side was covered at one end in one room, as well as the other half of the opposite side at the other end in the other room. Hut 16 had the end walls painted, as well as partition wall in the middle. In hut 17 the whole of one side wall and the roof above it were sprayed.

Periodical knock-downs were then made afterwards with the following results —

TABLE

NO. IN. KNOCKED 18,747 WITH 4.6 PER CENT SOLUTION DDT IN MARCH

	Hut 13	Hut 14 (Control)	Hut 15	Hut 16	Hut 17
Before spraying					
<i>A. funestus</i>	469	121	1,433	401	181
<i>A. gambiae</i>	4	4	3	3	1
<i>C. fatigans</i>	10	6	23	14	6
10 days after spraying					
<i>A. funestus</i>	Nd	133	Nd	Nd	Nd
<i>A. gambiae</i>		2			
<i>C. fatigans</i>		2			
28 days after spraying					
<i>A. funestus</i>		13			
<i>A. gambiae</i>		1			
<i>C. fatigans</i>		6			
30 days after spraying					
<i>A. funestus</i>		12			
<i>A. gambiae</i>		1			
<i>C. fatigans</i>		0			

It will be seen from the above table that the treatment of only one wall out of four in the rooms of hut 15 has been sufficient to bring down the number of anophelines from 1 155 to nil, and that the rooms continue to be anopheline-free 50 days after treatment. The same conditions obtain in the other huts.

Before proceeding with the knock-downs lengths of white calico are placed side by side on the floor so as to cover it up completely. Tops of tables and of other pieces of furniture are also treated in the same way. The habitation is then closed and treated with "pyreth" spray which is allowed to act for 15 minutes, after which the fallen insects which are easily seen on the white background and can hardly be missed, are counted.

The experiment is being continued on a larger scale, and although it has been going on for only just under 2 months the results are, I think, of sufficient interest to warrant their publication and it would be interesting to hear of similar experiments with other species under varying climatic conditions in other parts of the world.

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EFFECT OF CLIMATE ON THE BLOOD PRESSURE IN ACCLIMATIZED SUBJECTS

BY

L P R FOURMAN,*

From the Nuffield Department of Clinical Medicine, Oxford University

Evidence on the effect of climate on the blood pressure is conflicting (SUNDSTROEM, 1927) Physiological experiments have shown that the blood pressure falls with rise in environmental temperature (MCDOWALL, 1938), but such experiments have generally been made over short periods of time on subjects not accustomed to the effects of heat In acclimatized healthy young men, there is little change in blood pressure (EICHNA, BEAN, ASHE and NELSON, 1945) During a voyage from Mombasa through the Red Sea to the Mediterranean in early September, 1946, the opportunity presented to observe the blood pressure changes that might occur in subjects of various ages who are acclimatized to the effects of heat We hoped also to see whether under the hot and humid conditions obtaining in the Red Sea any cases of hypertension might be masked by a fall in blood pressure Findings in the Red Sea were compared with observations made 3 days later in the relatively comfortable climate of the Mediterranean

METHOD OF INVESTIGATION

The subjects were male Italian civilian internees from Entrea who had spent the war years in the tropics and who for various reasons were being repatriated in a hospital ship Their ages varied from 25 to 70 Many were suffering from non-medical conditions without cardio-vascular disease No acutely ill patients were included in this study, which involved 13 normal subjects under 50 years of age (eight under 30), 22 subjects over 50, 10 with miscellaneous renal diseases but without hypertension (six with renal stone, three with nephritis and one with pyuria), and three patients with hypertension Except in the patients with hypertension, no evidence of cardiac enlargement or retinal arteriolosclerosis was found, but many of the patients

* Thanks are due to Lt -Col B MEASHAM, O C , Troops, H S "Oxfordshire," for permission to make these observations

over 50 had arteriosclerosis. Eight subjects in whom, on one or other occasion, no diastolic reading could be obtained were not included in the analysis. Every effort was made to ensure a liberal fluid and salt intake, and the urinary chloride content was determined by the Fantus test in all subjects. In three the urinary chloride was less than 4 grammes per litre, and with increased salt intake their blood pressure rose from low values. These results have not been included in the analysis.

During the first set of observations the wet and dry bulb thermometer readings at 5 a.m. in the wards were 88° and 64° F respectively during the second set the corresponding readings were 75° and 85° F. This represents a change of about 20 per cent. in the relative humidity for the two sets of observations. Subjectively the change in temperature was one from great discomfort to tolerable comfort. Day temperatures were higher even on deck where the patients spent most of their waking hours. All blood pressure readings were taken between 5 a.m. and 6 a.m. before the patients had risen from their night's sleep and therefore as near as possible under basal conditions.

RESULTS AND CONCLUSION.

The results of the analysis of the two sets of observations are shown in Table I except for data in the patients with hypertension which are given individually in Table II. There was no significant change in the blood pressure in any of the groups.

TABLE I

COMPARISON OF BLOOD PRESSURES TAKEN IN THE RED SEA AND THE MEDITERRANEAN 3 DAYS INTERVAL

Group.	Number in group.	Series of observations.	Systolic B.P. mean and standard deviation.	Systolic B.P. mean of differences for each subject and standard error of mean.	Diastolic B.P. mean and standard deviation.	Diastolic B.P. mean of differences for each subject, and standard error of mean.
Normals under 50	13	Red Sea	100.2 ± 15.4	3.2 ± 4.0	67.9 ± 1.5	3.4 ± 3.5
		Mediterranean	99.0 ± 8.5		70.2 ± 5.6	
Subjects over 50	22	Red Sea	114.2 ± 18.9	0.2 ± 2.5	78.5 ± 8.5	8.6 ± 2.5
		Mediterranean	114.0 ± 18.8		73.5 ± 8.1	
Renal cases	10	Red Sea	106.0 ± 12.3	4.6 ± 4.3	73.4 ± 10.8	2.6 ± 3.4
		Mediterranean	103.4 ± 8.8		72.2 ± 11.0	

TABLE II
BLOOD PRESSURES IN THE RED SEA AND THE MEDITERRANEAN IN THREE PATIENTS WITH
HYPERTENSION

Patient	Observations	B P	
		Systolic.	Diastolic.
Essential hypertension	Red Sea	172	130
	Mediterranean	168	110
Polycystic kidneys	Red Sea	170	110
	Mediterranean	152	110
Pyelonephritis	Red Sea	174	130
	Mediterranean	152	110

These results indicate that in acclimatized subjects in hot climates a change in climate from one causing marked discomfort does not produce any consistent change in the blood pressure, provided changes due to dehydration are excluded. No masked cases of hypertension were discovered in the second series of observations.

SUMMARY

In sailing from the Red Sea to the Mediterranean, there is a sudden fall in temperature and humidity. Observations were made on the effect of this change in climate on the blood pressure. Forty-five subjects of varying ages and three patients with hypertension were studied under resting conditions. There was no significant change in the blood pressure when the relative humidity diminished by about 20 per cent.

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CHARLES MORLEY WENTON

President Royal Society of Tropical Medicine and Hygiene 1915-17
Honorary Secretary 1926-28

OBITUARY

C M WENYON, C M G , C B E , M B , B S , B S C , F R S

Dr Wenyon died suddenly at his home in London on Sunday, October 24th, just when he seemed to be recovering from a short illness. His end came peacefully and without pain, "And as one toyled with travaile downe doth lye, So lay he downe, as if to sleepe he went"

Charles Morley Wenyon was born in Liverpool in 1878. As a child he was taken to China, where his father was one of the pioneer medical missionaries, and came back to school in England at the age of fourteen. Having qualified in science at University College, London, and in medicine at Guy's Hospital, he was soon afterwards appointed Protozoologist to the London School of Tropical Medicine, thus entering on the course that was to lead to his eventual recognition as the foremost authority in medical protozoology throughout the world. He made special studies and investigations in the Sudan, and in Bagdad, Aleppo and Malta, and in 1914 he was appointed Director of Research in the Tropics to the Wellcome Bureau of Scientific Research. During the first World War he carried out a vast amount of research on protozoological infections, in Egypt, India, Mesopotamia and Macedonia. In 1924 he succeeded Andrew Balfour as Director-in-Chief of the Wellcome Bureau of Scientific Research, and finally retired from the Directorship of the Wellcome Research Institution in 1944, though he maintained his active association with the Wellcome Foundation to the last.

Wenyon's distinguished service to his country in the war of 1914-18 gained him the C M G and C B E. His eminence in the world of science was acknowledged in the award of many honours. He was a Fellow of the Royal Society, an Officer of the Legion of Honour, an Honorary Life Member of the New York Academy of Sciences, an Honorary Fellow of the Royal Society of Medicine, and the recipient of the Theobald Smith Gold Medal, and the Manson Medal.

Although Wenyon's work was mainly devoted to protozoological problems with some direct bearing on tropical medicine, it was by no means restricted to this field, and it can be said that there is hardly a single group of parasitic protozoa with which his name is not associated. Wenyon was the first to sort

out the intestinal protozoa of man, and to bring order out of the pre-existent confusion. He described *Endamoeba nana*, and the cysts of *Iodamoeba butschlii* and much of the present knowledge of the intestinal flagellates of man is owed to him. His work on *Leishmaniasis* threw light on the epidemiology and diagnosis of this group of diseases. He showed that *Leishmania* can pass into a leptomonad stage in a blood-sucking insect, and first demonstrated leptomonad flagellates in *Phlebotomus*. Wenyon himself appreciated the significance of this latter discovery but, through a cruel trick of fortune an experiment designed to prove the truth of his conviction miscarried, and could not be repeated. His studies on another group of haemoflagellates the Trypanosomidae, added materially to the sum of knowledge of these parasites and the diseases they bring about and his rational classification of the family has been generally adopted. He was the first to describe the exogenous development of the coccidium of man, and established the standing of this species through his extensive studies on the coccidia of dogs and cats. The differing response of the species of malaria parasites to quinine, the seasonal incidence of the disease, the development of the oöcyst in hibernating mosquitoes, were all investigated with characteristic thoroughness, and some of his findings helped to account for the strange vernal outburst of relapses of benign malaria, the "Spring-ill" of the early English. The classic manual *Protozoology* stands as a monument to his life's work.

Wenyon was a man of outstanding, even unique, personality. His boundless vitality served to fire his own enthusiasm, and, overflowing in some rustic fashion, transferred its influence to all with whom he came into contact. Stories are told of workers in remote laboratories, disappointed and sick at heart. When Wenyon, in his office of Consultant to the Army arrived, it was like a breeze blowing from a heathery hill through a vitiated and stagnant atmosphere, and on departing he left behind him a lasting and unforgettable inspiration. He was transparently honest and inherently incapable of any mean or petty act. Free himself from any thought of self-aggrandizement, he was untrusting generous in helping the deserving who turned to him for aid—but he did not suffer fools gladly and the pretentious poseur he could not endure. His vast professional erudition and experience in the laboratory and in the field, made him a trusted counsellor even in matters beyond the range of his own speciality and whatever he said or did was imbued with wise judgment and native common sense.

Wenyon's death has left a void in this Society which he loved and worked for so long in the great world of tropical medicine, and above all in that speciality which his own labours served so largely to create.

The Englished version of Johnson's famous epitaph on Goldsmith, with one term replaced, might serve equally for Wenyon. There is almost nothing in his Science that he did not touch, and he touched nothing that he did not adorn.

CORRESPONDENCE.

BANCROFTIAN FILARIASIS

To the Editor, TRANSACTIONS of the Royal Society of Tropical Medicine and Hygiene

SIR,

As one of the many who must work remote from medical libraries, I envy Lt-Col CLAYTON LANE the wealth of references listed in his paper on bancroftian filariasis (*Trans R Soc trop Med Hyg*, 41, 717) My comments will inevitably suffer from this lack of reference, and are based mainly on the points made in his paper

His more important conclusions are —

“ In the periodic type of infection the nightly rise in the microfilarial blood tide is due primarily to synchronized parturitions by the female worms, which probably precede the rise of the tide by a few minutes

Provided they are not overtaxed, this rise is modified or annulled by the active cells of the host's macrophage system recruited in the lymph tract and lungs

The fall of the microfilarial blood tide is due to the destruction of the nightly brood in liver, spleen and adrenals, probably in lymph nodes and possibly elsewhere ”

The other conclusions are subsidiary to this main theme of regular synchronized nightly parturitions and daily destruction of microfilariae, which certainly provides a satisfying explanation of the phenomenon of microfilarial periodicity in the blood But some doubts arise when one sets out the evidence in tabular form as shown

One must agree that there is evidence that all female worms in any one host are in approximately the same stage of pregnancy, there is also evidence that parturition, when it occurs, is expulsive and empties the uterus to a certain level, what is still doubtful is the gestational time-table, and a satisfactory link with the blood periodicity With a 24-hour cycle, I would expect to find much

closer correspondence between stage of pregnancy and time of day. Rod., whose worms had empty uterine stems at 14.00 hours, is now suggested as an example of abnormal parturition, but this immediately raises the rather awkward question of how many of the other samples might also have been from cases with abnormal worm parturition and the timing of the blood infection is scarcely mentioned. Conclusive proof of nightly parturition has yet to be found the facts could equally well be explained by the existence of a much longer period of gestation, expressed in days or even weeks, but combined with

Case.	Time results.	Blood results.	Material examined.	Findings in lymph nodes.	Findings in uterine stems.	Stage of pregnancy suggested.
Bel.	26 hrs. ?	Not started	Testis, cord and hydro- cele	Many free inf.	Larvae turbulent	Almost refilled?
L.Q.	00.40- 11.00	Not started	Testes and cords	No inf. found	Embryos out stretched	Almost refilled
Mil.	02.40- 03.03	71 inf. just before death	1 g 1 nodes	ML, con- b y and eggs	Embryos stretch- ed and parallel	Partially almost due
Ric.	11.18	1035 inf. in night blood	Epirochlear and tegu- sal nodes	ML, con- b y and eggs	Indefinite	Almost refilled?
Cor.	11.30	Not started	1 g 1 nodes	ML, con- b y and eggs	Larvae stretched but interlacing	Almost refilled
Rod.	14.00	Not started	1 g 1 nodes	Not started	Almost empty	Just before parturition
h.n.	14.43	ML pre- sent	1 g 1 nodes	Few inf in stool	Larvae stretched but interlacing	Almost refilled

a fair degree of synchronization between the adult worms, and with expulsive parturition

Except for the details of the rate of passage of drugs through the lymphatics, there is no evidence for the statement that the microfilariae reach the blood within a few minutes after parturition. Indeed, on page 724 two cases of MASON'S are mentioned in which a periodicity of microfilariae in lymph was emphatically present it differed from that in the blood by having a morning peak.

Both these cases showed signs of obstruction to lymph flow otherwise one might be tempted to suggest the lymphatic system as the daytime hiding place of microfilariae. All that one can say is that these, and other cases mentioned, showed considerable time intervals between the appearances of microfilariae in lymph and in blood.

CORRESPONDENCE

Whether there is a time-lag or not, CLAYTON LANE suggests that active destruction of microfilariae goes on in the lymphatic tract, that a normal larvicidal barrier, sufficient to modify or annul the rise of the blood tide, exists between the adult female worm and the peripheral circulation. For the evidence of the existence of this barrier to be fully convincing, I think that it would have to be obtained from a person who had not yet developed signs of obstruction to lymph flow. Of all the patients mentioned, apparently Ric is the only one to satisfy this condition, the others had varicose lymphatics, hydrocele, or other signs of lymphatic obstruction in varying degree. Indications of larvicidal macrophage activity in such material can scarcely be accepted as typical of what occurs in the early stages of the infection.

Various specimens show proof of the destruction of microfilariae in liver and spleen, and this is held to be the mechanism responsible for the daily fall of the blood tide. There can be little doubt of the fact of destruction, but there may be some doubts as to the rate at which it occurs. The degenerating microfilariae found in these visceral sections may represent several days' gleanings from the blood stream.

Perhaps, however, one of the greatest obstacles to acceptance of the idea of nightly worm parturition is a study of the results obtained by CULBERTSON (1947) and his fellow workers in the drug treatment of filariasis (*Trans R Soc trop Med Hyg*, 41, 18). They themselves interpret their results as indicating that the drugs acted chiefly on the adult worms, and had very little direct effect on the microfilariae, despite the early killing or sterilization of the adult worm, microfilariae may persist in the blood for several months, but having once disappeared they seldom returned. No other interpretation would seem to fit the facts so well. On the other hand, SANTIAGO-STEVENSON and others (1947), reporting on the new drug "hetrazan" (*J Amer med Ass*, 135, 708) explain its action in rapidly clearing the blood of microfilariae by saying that its primary effect is on the microfilariae, but that it also has some effect on the adult worms.

I may summarize these comments by expressing my opinion that the evidence in favour of nightly birth and daily death of microfilariae is not sufficiently conclusive, there is evidence that they may enjoy a much longer life, and in fact the pregnancy cycle of *Wuchereria bancrofti* has yet to be established. CLAYTON LANE has pointed out very clearly what details require special attention when material for this purpose is being collected.

I should like to refer briefly to two side issues. I was very interested to find that the youngest reported blood infection with *W. bancrofti* was a child of 14 months, and the suggestion that intra-uterine infection of an infant before birth was theoretically possible. I have notes of a blood survey made last year in a rural area of Malaya, in which the youngest child with a blood infection of *W. malayi* was aged exactly 13 months. (Date of birth 13 4 46, date of survey, 12 5 47). This finding was doubted at the time, because the parents originally gave the age as 9 months, but was confirmed by repeating the blood

film the correct age was obtained from the register of births. The father had an elephantiasis of both legs of 10 years duration the mother had microfilariae in the blood but no symptoms and a brother aged 3 years, had no sign of infection. Interesting, but inconclusive.

Secondly it seems to be a universal finding that persons with developed elephantiasis seldom show microfilariae in their blood. This can be explained by supposing the adults to have been either killed or shut off from the general circulation during the attacks of lymphangitis which usually accompany the development of the deformity but how is one to explain the apparent lack of reinfection? It seems unlikely that worms acquired after the onset of elephantiasis should all be trapped in the obstructed lymphatics one would imagine that some would find their way into unobstructed channels, and that the blood infection rate of persons with elephantiasis in an endemic area would not be very different from that of other persons in the same area whereas it is usually very much lower. If there is a defensive mechanism at work, which either prevents the infective larvae from growing to adults, or keeps the adults sterile it must have some connection with the attacks of lymphangitis. I do not remember having seen any explanation of this particular problem, and would be grateful for enlightenment.

I am, etc.

T. WILSON

Sungei Patani
Malaya.

DR C M WENYON AND THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE

By Sir PHILIP MANSON-BAHR, *President*

Having had the privilege of nearly forty years' friendship with Dr Wenyon, and the rare good fortune of sitting at his feet, it has now become my duty to record his services to the Royal Society of Tropical Medicine and Hygiene. He served as Honorary Secretary for quarter of a century (1920-1945), an achievement crowned by his Presidency from 1945-1947.

In 1921 he was joined in his secretarial work by his sister, Miss Mildred Wenyon, and together they formed an ideal partnership. With such fidelity did he carry out his duties that he must have attended over one thousand committee meetings, and he rarely missed the appointed hour.

When he succeeded Dr G Carmichael Low as Honorary Secretary, the Society's headquarters were situated in a room of The Medical Society of London, 11, Chandos Street, where its activities were continued for the next twelve years. During that time the scheme for acquiring a permanent home in memory of Sir Patrick Manson was launched under the aegis of Sir Andrew Balfour with the guidance of Dr Wenyon as Honorary Secretary and Sir Arthur Bagshawe as Treasurer. In spite of the generosity of Dr Low, his lifelong friend, and other Fellows of the Society, funds came in slowly so that at one time Wenyon and two of his associates offered themselves as sureties for a large loan from the Westminster Bank. The search for a suitable home proved a difficult matter, and for over five years he spent many hours on this fruitless task until he chanced on 26, Portland Place, a substantial building which possessed a suitable space for a hall, an area at that time occupied by derelict kitchen premises. We must now admire his vision in realising that, though the Society could not then occupy the whole of the building, the residential quarters constituted an asset destined to bring in an income which in course of time has more than trebled, and which now forms a solid financial background. The extent of the negotiations involved in these transactions was enormous, and in his dealings with lawyers, architects and others he showed a business acumen of a high order, and a clear grasp of finance. His dream came true when the first meeting of the Society took place in Manson House, 21st January, 1932. It was graced by a typical address on the transmission of leishmaniasis in its best and thorough style.

Eventually at a cost of over £30,000, a sum to which the present King and the Duke of Windsor subscribed, Manson House was officially opened by the latter (as Prince of Wales) on 17th March, 1932.

Many will remember Dr Wenyon a look of triumph when in May 1945 the President was able to announce that the loan had been paid off and that the Society was free from debt.

As Honorary Secretary almost one of his first undertakings was the re-organisation of the *Transactions*. Older Fellows will remember the little green pamphlet which in Vol. XV (1921-22) became transformed into the present grey covered imposing publication. Many of the papers published since that date bear the mark of his handiwork in rearranging, editing, and, at times, even transcribing. For this devotion many a humble contributor has had reason to be grateful. As has been said, he built up the *Transactions* as a model of editorial efficiency.

To Wenyon, too, we owe the institution of the bi-annual Laboratory meetings which have become such popular functions of the Society. It is also indebted to him for its crest of *Anopheles gambiae* and for obtaining that most appropriate motto "*Zona torrida intemera*."

Both the Chalmers and Manson medals, the chief awards of the Society were instituted under his guidance and with negotiations which were not always easy to conduct. Throughout his career Wenyon was spurred on by a desire to cater for the needs and wishes of the many Fellows abroad and to make their Fellowship worth while. When he took up his duties the number of Fellows was a little over 600 but before his Presidency this had risen to over 1 700, many of whom he himself had attracted. The success of this Society the popularity of its meetings and the quality of its communications were such that all interests in tropical medicine in the metropolis became centred in Manson House. In 1930 a Branch was opened in Edinburgh for the Fellows in that area where it has proved a great success.

May the Royal Society of Tropical Medicine and Hygiene long continue to flourish as a monument to the genius of Charles Morley Wenyon.

[The previous number of these Transactions, Vol 42, No 3,
was published on November 27th 1948]

TRANSACTIONS OF THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE

VOL 42 NO 4 JANUARY, 1949

LABORATORY MEETING

of the Society held at the

Liverpool School of Tropical Medicine, Pembroke Place, Liverpool, 3,

on

Thursday, 18th November, 1948, at 7 30 p m.

THE PRESIDENT,

SIR PHILIP MANSON-BAHR, C M G , D S O , M D , F R C P ,
in the Chair

Lord Leverhulme, Chairman of the Liverpool School of Tropical Medicine, gave an address of welcome to the Society. He said this was the 50th year of the School's activity, it having been founded by Mr (later Sir) ALFRED LEWIS JONES on 12th November, 1898, early the following year the staff was appointed and included the then Major RONALD ROSS as Lecturer in Tropical Diseases. Lord LEVERHULME referred to the early days when the School was dependent on Liverpool business men and on merchant and shipping houses, today the School is associated with the University, but the original ties remained strong of which there could be no finer proof than the munificent gift of £10,000 officially received on the 50th anniversary of the founding of the School, from the Liverpool firm of West African shippers, Messrs John Holt and Company. The late Mr ROBERT HOLT was a highly valued member of the School Council and the gift would always be associated with his name by those who remembered him. At the wish of the donors, the gift is to be devoted especially to the furtherance of research on malaria.

Lord LEVERHULME mentioned that this was the second meeting the Society had held in Liverpool since the war, and said he hoped visits from the Society would be repeated frequently.

DEMONSTRATIONS

LIVERPOOL SCHOOL OF TROPICAL MEDICINE.

DEPARTMENT OF TROPICAL MEDICINE.

Prof B O Macgregor, Dr W H Horner Andrews, Dr M. M Totley
Dr R H Townshead and Dr C E M Wenyon

Demonstration of techniques for biological research.

The demonstration was designed to illustrate some of the techniques used in the Department for the study of physiological and pathological processes in malaria in man and other animals. Techniques shown are listed below

- (1) Estimation of small quantities of sodium and potassium by means of flicker photometer devised in the Department (Dr C. E. M. WENYON)
- (2) Measurement of hepatic blood flow in the intact animal (Method depends on the use of radioactive P_{32} and was designed by H. B. JONES and E. L. DODSON *J. Clin. Invest.* 1944 23 783)
- (3) Modification of Warburg constant volume respirometer for measurement of dissociation of oxyhaemoglobin. By this method several points on dissociation curve can be found with as little as 1.0 ml. blood. This makes the method suitable for work on small animals. (Mr H. B. H. SCULLTHORPE.)
- (4) The use of the Warburg technique for the study of cytochrome-oxidase systems in malaria and for the investigation of the respiration of malarial parasites and the action of anti-malarial drugs on such respiration.
- (5) Biochemical techniques for the study of blood in malaria: determination of bicarbonate content (CONRY 1936), inorganic phosphate (DUNN-CURTIS, L. and CHAN E. 1935) chloride (SANTOIR 1937) potassium (KING E. J. 1946) sodium (KING E. J. 1946)
- (6) The use of aerial X-ray opaque metals for the study of drug action on the gastrointestinal tract of small animals and man. Demonstration of the action of mepacrine M.3349 spomorphine atropine and other compounds. (With G. M. BROWN Army Malaria Research Unit, 1948.)
- (7) The liver in malaria and other conditions.
The similarity of the hepatic lesions in shock due to many different agents, including malarial tertian malaria and blackwater fever was shown by means of sections and photography.
- (8) The pathogenesis of centrilobular necrosis in the liver
Charts were demonstrated showing how centrilobular changes found in the liver may be due to anoxia, of anoxic stagnant or anoxicemic origin.
- (9) Perfusion of the liver
The apparatus demonstrated is for perfusion of isolated livers of small animals with simultaneous recordings of venous flow and volume changes. It consists essentially of copper and perspex constant temperature perfusion chamber with an arrangement for perfusing oxygenated glucose Locke solution containing 3 per cent gum arabic at constant pressure.
- (10) Investigation of the calibre of the hepatic sinusoids and portal vein
The method consists of injecting sodium ink under anaesthesia into the splenic system, and cutting sections of 3/ys after the liver has been fixed *in situ*. The sections are dehydrated, and cleared in benzyl benzoate or oil of wintergreen.
- (11) (With M. A. H. SAUNDERS.) Demonstration of the point of meeting of the hepatic artery and the portal vein.

Solutions of diazo salt of 2-amino anthraquinone in normal saline and 2-naphthol-6-sulphonic acid in saline and sodium carbonate are used. One solution is perfused down the hepatic artery, the other down the portal vein. The point of meeting is indicated by formation of a brick red gelatinous precipitate which remains *in situ*.

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Dr M M Tottey and Dr R H Black

Respiration of malaria parasites

The oxygen uptake of malaria parasites can readily be measured in the Warburg apparatus. One ml oxalated parasitized blood and 2 ml serum are pipetted into the flask with a small amount of strong caustic potash in the central well, and after connecting with the manometer the flask is placed in the water bath at 37° to 38°. When equilibrium has been attained the manometer fluid is levelled and the flasks shaken. The red cells and parasites take up oxygen and the carbon dioxide produced is absorbed by the potash. Any reduction in the volume of gas in the manometer therefore represents the volume of oxygen consumed. If a similar flask is set up containing oxalated non-parasitized blood it is found that the oxygen consumption is not nearly so great as it is for parasitized blood. If the red cell content of each flask is known the oxygen used by the normal erythrocytes in the infected blood can be allowed for and the amount of oxygen used by the parasites themselves calculated.

COGGESHALL and MAIER (1941) employed a similar technique in the development of a technique for the assessment of anti-malarial activity in new drugs. Instead of serum they suspended the parasitized blood in phosphate buffer and then added quinine or any other drug under test from the side arm of the flask. The reduction in oxygen consumption between this and the control flask to which no drug had been added was then measured. Unfortunately their results are of no value since they used amounts of drug which created concentrations which were 100 to 200 times as great as those ever observed after administration of the drug to animals. We have also observed that the concentration of quinine used by them entirely arrested the respiration of normal red cells.

In these laboratories we have attempted to detect any interference with the normal oxygen uptake of parasitized blood by known anti-malarial drugs added in physiological concentration. It has been proposed by HAWKING and by BLACK that mepacrine and paludrine exist in the blood stream in some modified form and that only these forms are active against the parasites. We

have therefore tested paludrine, mepactine and quinine both by adding a solution of it to the parasitized red cell suspension in serum and by using serum taken from birds which have had very high doses of the drug. In no case have we detected any effect upon the oxygen consumption of the parasites suspended in such a medium.

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Dr R. H. Townshend.

Failure of an attempt to demonstrate hyaluronidase production by *Entamoeba histolytica*.

The ability of *E. histolytica* to penetrate the mucosa of the intestine and spread in the tissues is presumably due to enzymes produced locally by the amoebae. Such enzymes may break down the cells themselves or may attack the intercellular cement and allow the cells to be separated. Hyaluronidase, an enzyme produced by some bacteria, acts in the latter manner: an attempt was therefore made to demonstrate the presence of this enzyme in cultures of *E. histolytica*. The amoebae were grown in a simple fluid medium with rice starch, and the culture fluid was centrifuged and tested for hyaluronidase by the viscosimetric method of Swyer and Emmers.

Many of the cultures contained hyaluronidase and at first it was thought that the amoebae themselves were producing the enzyme because control cultures with bacteria alone showed no enzyme production. Later controls were incubated anaerobically however and much hyaluronidase was produced and was shown to be associated with the growth of *CL. welchii* in the medium. *CL. welchii* is well known to be a hyaluronidase forming organism and grows in the anaerobic conditions produced by *E. histolytica* at the bottom of the culture tubes.

That hyaluronidase was produced only in tubes containing the amoebae was due to the fact that only then was complete anaerobiosis obtained.

It is thus clear that hyaluronidase is not formed by *E. histolytica* but it is still possible that the penetration and spread of the amoebae may be facilitated by hyaluronidase produced by *CL. welchii* and other anaerobes growing in close association with the amoebae in the local area of anaerobiosis which is there obtained.

DEPARTMENT OF CLINICAL MEDICINE

Dr A. R. D. Adams and Dr D. R. Seaton

- 1 Resistance to paludrine developed by a strain of *Plasmodium falciparum*.
- 2 Photographs of a European case of lepromatous leprosy treated with sulphatrene.

1 A West African strain of *P. falciparum* has been maintained by serial

blood inoculations for the past 12 months. During this time it has been constantly exposed to paludrine treatment. Its resistance to paludrine has steadily increased and at the tenth passage asexual parasites were present in blood films throughout a 10-day course of 1 gramme of paludrine daily.

2. Colour photographs of a case of lepromatous leprosy before and after 6 months' sulphetrone treatment were shown, also photographs of erythema nodosum developing during sulphone therapy.

Dr E. M. Lourie and Dr D. R. Seaton

Resistance to paludrine developed by a strain of *Plasmodium vivax*

Charts and tables were shown demonstrating the acquisition of paludrine-resistance by a strain of *P. vivax* as a result of treatment over a period of 18 months in a series of trophozoite inoculated patients. Details will be given in a forthcoming publication.

DEPARTMENT OF ENTOMOLOGY AND PARASITOLOGY

Prof R. M. Gordon, Dr L. J. Chwatt and Dr C. M. Jones

1. The breeding places of *Chrysops* in the British Cameroons
2. The infection rate with *Loa loa* in the fly population, and in the human population at Kumba, British Cameroons

1. The difficulty of planning measures to reduce the incidence of loiasis without knowledge of the breeding places of the vectors is obvious. Until 1947, the breeding places of *Chrysops silacea* and *C. dimidiata* were unknown, but in June of that year, one of us (L. J. C.) reported the finding of a pupa containing a recognizable adult of *C. silacea* at Kumba, British Cameroons. A more thorough search of the area was carried out by the authors in June and July, 1948, and larvae of *Tabanus*, *Haematopota*, and, finally, *Chrysops* were found. Eleven breeding places of *Chrysops* were discovered in streams running along the bottoms of thickly overgrown ravines.

A map, and photographs of typical breeding places, were exhibited, showing that *Chrysops* larvae are restricted to certain habitats in densely shaded streams, where slowly moving water passes over a layer of mud covered with decaying vegetation. Photographs of the collecting of larvae (by washing the mud through a series of graduated sieves), and specimens of the larvae found, were also exhibited.

2. It is well known that only a proportion of persons suffering from malaria are, at any one time, capable of infecting the insect vector, while it is generally considered that in persons with loiasis the infective larvae are more or less constantly present throughout the hours of daylight. A table was exhibited which summarized existing information, and showed that, in the infected area, the proportion of the human population showing microfilariae of *L. loa* in the peripheral blood is much lower than would be expected from a

consideration of the infection rate in the *Chrysops* population, and also that evidence of lousis is often noted in persons without microfilaria in the blood. It follows that the absence of larvae from the peripheral blood is not necessarily a proof of the absence of living adults, and that the assessment of control and curative measures is, to a considerable extent, dependent on the discovery of a more reliable method of diagnosis.

Slides showing larvae of *L. koe* in the thorax, head, and mouthparts of *Chrysops* were also exhibited

Prof R M Gordon and Mr W Crews

- 1 Pool feeding by blood-sucking insects.
- 2 Sensitization to the bite of the tsetse fly

1 The employment of a flexible proboscis to lacerate the host tissues over a wide area, and so to produce a haemorrhage from which the insect can feed, was shown to be characteristic of a variety of blood-sucking insects. The sections exhibited showed the small haemorrhages following the bites of two species of mosquito and of *Cimex* and the more extensive haemorrhages following the bites of *Glossina* and *Chrysops*. A similar haemorrhage has been shown by SHORR and SWAMINATHI (1923) to follow the bites of *Phlebotomus*.

Such a method of feeding will influence the taking up of any parasites by the insect and may determine the site of deposition of any introduced parasites. During pool feeding many and in some instances the vast majority of the introduced parasites will be delivered into the tissues or into the blood pool, and not directly into the circulation, and it is suggested that the early development of such locally introduced parasites may be further affected either by the local reactions of the host to the insect's salivary secretions or by the normal cellular reactions of the wounded tissue.

2 It is generally accepted that marked reactions to uncomplicated tsetse bites, and also to the bites of other insects, are due to sensitization, but it is not so widely realized that individuals who have not become sensitized usually fail to exhibit even the faintest trace of a reaction. The exposure of 18 persons in this country to the bites of imported tsetse flies in no instance resulted in a reaction, whereas the further exposure of two of these caused them to become sensitized and to react violently.

The first individual experienced four bites within 2 weeks, and on no occasion showed a reaction. Six further bites, experienced simultaneously and within 4 weeks of the first bite, gave rise to a severe oedematous reaction, and occasional bites during the following year each gave rise to a very severe immediate reaction, with wheals as much as 3 cm. across and very pronounced swelling of the bitten arm for up to 7 days after the exposure. The second

One of these individuals showed slight erythematous reaction about 18 hours after the bite but it seems probable that this was caused by some introduced extraneous infection.

individual experienced five single bites at approximately fortnightly intervals, the first three bites producing no reaction, the fourth a slight immediate reaction, and the fifth a severe immediate reaction with a large wheal and pronounced erythema of the surrounding area of the arm

The exhibited colour photograph, which was taken immediately after a tsetse fly was allowed to feed on the first individual, showed the wheal, which had not reached its full size. Also exhibited was a section through the bitten area of the skin of the same individual, taken when the wheal was about the size shown in the photograph. In addition to the usual large subcutaneous haemorrhage, the sectioned tissues showed dilatation of the blood vessels and marked separation of the collagen fibres. A similar histological picture was shown in the case of a rabbit which had been passively sensitized by serum from this individual.

Mr W Crewe and Prof B G Maegraith

A case of severe generalized reaction to mosquito bites

It is well known that in certain instances severe generalized manifestations may occur in sensitized subjects as the result of bee and wasp stings, but such general reactions are seldom reported following the bites of blood-sucking insects. A reaction of this type was, however, exhibited by a patient suffering from falciparum malaria who, in an attempt to infect mosquitoes, was exposed to the bites of 380 *Anopheles maculipennis* and 20 *A. stephensi*.

The clinical report on this patient was as follows

McK Merchant Navy Aged 39

Overseas service 1925-30, 1940-48 China, Singapore, India, Burma, West Africa. History of several previous attacks of malaria. Admitted 5.8.48 suffering from falciparum malaria acquired in West Africa. Treated with paludrine 300 mg b.i.d. for 10 days. Asexual parasites absent from blood by third day of treatment. Gametocytes persisted. *Entamoeba histolytica* cysts present in stool. Treated with standard 3 weeks' course. After treatment for amoebiasis, falciparum gametocytes were still present and it was decided to attempt to infect *A. maculipennis* and *A. stephensi*.

7.9.48—Four hundred mosquitoes (*A. maculipennis* except for 20 *A. stephensi*) were fed on patient's thighs in 28 minutes. Most mosquitoes fed. (No mosquitoes became infected.)

Ten minutes after feeding began, patient noticed epigastric pain followed by "flushing" radiating upwards to back of neck and down arms and later legs. He felt his heart pounding and noticed his vision was blurred. Large itching wheals developed on both thighs where feeding had taken place—more pronounced on left, where *A. stephensi* had fed. Wheals also appeared on neck and arms, particularly at the wrist and flexure of the elbow joints. The skin of the face, including the conjunctival vessels, the trunk, arms and legs became deeply flushed.

At this stage the pulse rate was 150 per min., the pulse full and bounding, and the blood pressure 80/40. There was no appreciable rise of temperature. Stroking of the skin failed to elicit a white line response.

The administration of anthisan 0.1 gramme orally was followed by marked improvement associated with a slowing of the pulse rate to 90 per min. and a rise in blood pressure to 120/70. Flushing of the skin faded in 3 hours except over the region of feeding. A further dose of anthisan 0.1 gramme was given 5 hours later. The wheals in the feeding area persisted for 24 hours. Two days later there were numerous small red papules scattered over the site of the mosquito bites. The patient was well except for occasional headaches.

Some 3 weeks later this patient volunteered to undergo a test to determine whether he was abnormally sensitive to *Anopheles* and whether he was similarly sensitive to the bites of other genera of mosquitoes. He was exposed, on the flexor surface of the left forearm, to one specimen of each of *A. maculipennis*, *A. stephensi*, *Aedes aegypti* and *Culex molestus*. All four mosquitoes became gorged at approximately the same time, and the patient reacted to all bites within a few minutes.

Three colour photographs of his subsequent reactions were exhibited. The first was taken about 5 minutes after the bites, and showed a large wheal resulting from each bite, with an extensive area of surrounding erythema. The second, taken 1 hour later showed a reduction both in the size of the wheals and in the area of erythema, while the third, taken 24 hours after the second, showed the site of each bite marked by a small red papule, with some erythema surrounding the *Aedes* bite.

This individual thus showed a not exceptionally severe, and approximately equal, reaction to the bites of these four species of mosquito, the very severe generalized reaction originally experienced being apparently caused by the sudden injection of an unusually large dose of antigen (by 400 mosquitoes feeding simultaneously).

Dr W. E. Korahaw

Some observations on *Leishmanoides carinii* (Travassos 1919) Chandler 1931

(1) Development of the first stage larva

The morphological development of the first stage larva of *L. carinii* was shown by means of photomicrographs, slides and frequency distribution curves of the lengths of the larvae. The course was followed from the shedding of the vitelline membrane at birth through an increasing complexity of nuclear architecture with a small successive increase in length occurring on their migration to the pleural sac, and finally on their fairly rapid progress to the peripheral circulation. It was shown that the less complex larvae in the pleura are unsheathed, and that in some of those in the peripheral circulation sheaths can be demonstrated easily but in others with the greatest difficulty or not at all. Sheathed or unsheathed, the morphological development of those in the blood is uniform, and the presence or absence of a sheath cannot be taken as the sole evidence of maturity.

The demonstration showed the early larval stages of *L. carinii* for comparison with such forms as are known of the corresponding stages of *Wuchereria bancrofti* and *Loa loa*.

Dr D. S. Bertram.

A method of inducing controlled infection with *Leishmanoides carinii* in the cotton rat.

The exhibit dealt with the intensity of infections obtained in batches of *Lepus sylvaticus* mites recovered from cotton rats infected with *L. carinii*.

Individual mites usually contain small numbers of infective worms, but as many as 67 infective forms have been found in a single mite. Infection rates of from 11 per cent to 86 per cent have been obtained. By obtaining mites with high infections it is possible to use small numbers of the mites (about 20) to infect a cotton rat. Data were shown indicating that, if no gorged mites are recovered from a rat exposed to infective mites for 24 hours, the rat was unlikely to become infected, but that if some gorged mites were recovered the expectancy of the host becoming positive for microfilariae was high (about 95 per cent successful infections).

The conclusions were made that, in studies on chemoprophylaxis, the recovery of gorged mites was necessary on the occasion of exposing the rat to infection and that on account of the variable numbers of worms found in mites of the same group, the number of worms transmitted to a rat by a known number of mites could be estimated only within very wide limits.

Dr W E Kershaw, Dr D S Bertram and Mr J Williamson

The chemoprophylaxis of filariasis in the cotton rat

The trial of drugs as prophylactics against the development of filariasis in the cotton rat was undertaken by administering the drugs in solution by injection into the peritoneal cavity, then after varying intervals, subjecting the animals to the bite of infected mites, by the method devised to induce a controlled infection, and allowing the infections to develop and run their course.

The rats were subjected to infection in five groups of eight, each group consisting of two controls and three pairs which had been given MSb (Friedheim), stilbamidine and antrypol.

The exhibit consisted of charts showing the results of exposure to infection, and where infection had been acquired its course was estimated by the count of microfilariae in the peripheral circulation. Of the ten rats which had been given MSb (Friedheim), eight failed to develop microfilariae in the peripheral circulation, one died immediately after exposure to infective mites, and microfilariae were found in the blood of the other. In this single exception, the concentration of drug in the blood, as measured by the anti-trypanocidal titre, was lower than the others. From the course of the other two groups of rats which had received drugs, neither stilbamidine or antrypol appeared to act as a prophylactic.

Dr D S Bertram, Dr W E Kershaw, and Mr J Williamson

The course of untreated infections of *Litomosoides carini* in the cotton rat and the application of the observations made to chemotherapeutic treatment.

The necessity for caution in interpreting the results of chemotherapy in naturally infected cotton rats has already been stressed.

The demonstration showed the course of an untreated infection, from exposure of the rat to infected mites to the appearance of microfilariae in

peripheral circulation some 7 weeks later. The count rose to a more or less steady level during the next 2 months, and was maintained at that level for a further 3 or 4 months. A gradual fall lasting 5 months then set in until the microfilariae finally disappeared from the peripheral circulation some 11 months after their first appearance. It would appear from these observations that the only reliable period for chemotherapeutic trial is during the first 3 or 4 months invasion.

The survival of microfilariae in transfused blood which in the particular experiment shown was about 1 month, was shown to have a rate of fall similar to that of an infection undergoing spontaneous cure.

From the course of the infections in the rats observed in this laboratory it would appear that the infection can terminate in two ways

1. The adults may die and become absorbed, the microfilariae remaining in the circulation for considerably longer period, until they can be no longer found in thick blood film.

2. The output of microfilariae may be reduced, the survivors reaching the peripheral circulation in numbers insufficient to be found in thick blood films, though live adults and few active larvae may still be found in the pleura.

Examples of both these methods of termination were demonstrated.

Miss E. W. Roberts

Slides showing the miracidia of *Fasciola hepatica* in the foot and mantle of *Lymnaea truncatula*, one hour after exposure to infection

The presence of miracidia of the schistosomes in the tissues of their molluscan intermediate host has been demonstrated by GORDON DAVEY and PRANTON (1934) and BRUSTERT (1940), and others. BACIGALUPO (1933) showed the presence of miracidia of *F. hepatica* in the mantle of *Lymnaea stagnalis*. As far as we are aware the presence of the miracidia of *F. hepatica* in the tissues of *L. truncatula* has not previously been shown.

Laboratory bed snails about 1.5 mm. in length were exposed to 50 newly hatched miracidia in a small quantity of water. After 1 hour the snails were removed, killed and fixed immediately in Bouin's fluid. The sections were cut at a thickness of 7 μ and stained in Delafield's haematoxylin and eosin.

LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE

DEPARTMENT OF ENTOMOLOGY

Dr W. A. Lamborn (introduced by Prof P. A. Buxton).

Remarkable mud columns in which the larva of *Tuberosa bipunctatus pupatus*.

In Nyasaland *T. beguittatus* deposits its eggs in shallow temporary pools. When such a pool dries the mud cracks with a large mesh. But at places where the cracks might be expected to meet one may find a round mud disc, which is in fact the top of a column of mud.

It is surmised that the column of mud is isolated by the full grown larva when the pond is drying up and the mud becomes semi-solid. It seems that the larva starts at the surface of the mud and goes downwards in a tight spiral, isolating the column which can be as much as 6 inches long. It then returns nearly to the top on a much looser spiral, as seen by examining the outside of the column. When it has nearly reached the top it turns inwards and excavates a cell in the column in which it becomes a pupa. The pupa, as it appears, scrapes its way through the top of the column before the emergence of the fly occurs (W A LAMBORN, *Proc roy Soc*, Series B, 106, 1930)

DEPARTMENT OF PARASITOLOGY

Prof H E Shortt

- 1 Diagram of life-history of *Plasmodium cynomolgi* with actual specimens of the various stages under the microscope
- 2 *Plasmodium berghei* Malarial parasite of the tree rat in the Belgian Congo (slide presented by Dr VAN HOOFF)
- 3 *Haemoproteus* sp in blood of the Gecko lizard (*Hemidactylus* sp)
- 4 *Haemogregarine* sp in blood of the Gecko lizard (*Hemidactylus* sp)
- 5 *Leishmania enriettii* Muniz and Medina New species described infecting the skin of the guinea pig in South America

1 Shows, in diagrammatic form, the complete life-cycle of a typical mammalian malarial parasite in the insect and vertebrate hosts. The diagram incorporates recent findings in connection with exo-erythrocytic development

Prof H E Shortt and Dr P C C Garnham

Earliest form seen to date of the pre-erythrocytic cycle of *Plasmodium cynomolgi*—fourth day after bite of the mosquito

The parasite is a small schizont, about 8μ in diameter, ovoid in shape, situated within a parenchyma cell of the liver. The entire schizont, as seen in serial sections, contains about 24 fragments of chromatin

Dr P C C Garnham

- 1 *Plasmodium* sp in the blood of East African skink, *Mabuia maculilabris* (from near Homa Mountain, Victoria Nyanza)
- 2 *Trypanosoma* sp in the blood of East African skink, *M. maculilabris* (from near Homa Mountain, Victoria Nyanza)

1 The smear showed asexual and sexual forms of a malarial parasite which occupies about half the red blood cell, not displacing the nucleus. Gametocytes are circular. Trophozoites may be very ragged in outline

2 This haemoflagellate exhibits the following characters. Posterior position in nucleus, complete twisting of the posterior extremity, presence of myonemes in the cytoplasm, a well developed undulating membrane, and a free flagellum

Approximate measurements are Length of body 30μ width, 8μ free flagellum, 9μ

Dr Dyson Blair (demonstration shown by Prof G Macdonald)

Series of 28 photographs on echistosomiasis in Southern Rhodesia

The 28 photographs exhibited were enlarged from the frames of a film which is now being made by the Government of Southern Rhodesia on echistosomiasis.

Pathological lesions were illustrated by photographs of the results of infection on the bladder and ureter and by microphotographs showing the tissue reaction to echistosome eggs in various parts of the body including the lung. Heavy deposits of eggs some with terminal spines, probably of *Schistosoma karmatobum*, were shown in the bladder wall in the neighbourhood of a rupture and other situations.

There were three photographs of the adult worm taken by dark ground illumination and a series showing the external development, including illustrations of the miracidium leaving the egg, of a sporocyst of cercariae leaving the snail's liver and of free living cercariae. There were also illustrations showing the reaction of the cercarial antigen skin test and of the methods used in the control of molluscs.

Dr D A Cannon.

Eggs of *Paragonimus* in sputum of patient in the Cameroons.

The sputum in which these eggs were found comes from a patient in the Cameroons who has a non-tuberculous chronic respiratory disease.

Records of paragonimiasis in Africa are extremely rare, hence this case is of interest. It is thought that there may be a considerable incidence of this infection in several parts of the Cameroons.

Mr Hilary Cross (Introduced by Prof J J C. Buckley)

- 1 Slides and photomicrographs illustrating the larval development of *Taenia taeniarformis* (*Cysticercus fasciolaris*) in the liver of infected rats.
- 2 Slides illustrating the process of transverse fission of *Cysticercus phillimhi* in experimentally infected rabbits.
- 3 Slide and photographs illustrating the difference in optical properties between blade and base of the rostellar hooks of taeniid cestodes (*Taenia serialis* and *T. taeniarformis*) when examined in polarised light.

1 (a) Section of liver with 10-days-old cysticercus.

(b) Section of liver showing a 25-days-old cysticercus with its scolex anlage (including rudiment of rostellum).

(c), (d) and () Sections through invaginated scolex anlagen of older larvae, showing further development of rostellum and the origin and growth of hooklets.

2 Slides illustrating the process of transverse fission of *cysticercus pisiformis* in experimentally infested rabbits

Transverse fission of *cysticercus pisiformis* begins in the liver and is most often completed there, i.e., before the bladder-worm migrates into the body-cavity. Fission involves an initial hypertrophy of the tissues (especially the parenchyma fibres) at the zone of constriction, followed by degeneration and loss of all the elements except muscle and parenchyma fibres which become consolidated into a slender fibrous cord binding the segments of the original bladder-worm. The rupture of this cord and the sealing-up of the broken ends of the segments complete the process. The anterior segment develops a scolex. The posterior segment or segments are acephalic. The majority of the bladder-worms do not undergo fission.

3 Slide and photographs illustrating the difference in optical properties between blade and base of the rostellar hooks of taenid cestodes (*Taenia serialis* and *T. taeniaeformis*), when examined in polarized light.

The blade of the hook is birefringent when examined in polarized light, while the base is isotropic. This is correlated with the fact that the base, which is composed of an unorientated scleroprotein, is more liable than the blade to intraspecific variation in shape and size. The blade, which is composed of an orientated protein-chitin, is a more constant structure within a species.

Sir Philip Manson-Bahr and Dr W E Cooke

Nasal smear in leprosy showing enormous concentration of bacilli from a European boy, aged 13, from Antigua

Dr H A Baylis, Sir Philip Manson-Bahr and Prof J J C Buckley

Demonstration of a cestode new in man—*Inermicapsifer arvicanthidis* (Kofend, 1917)—a parasite of rats in Kenya

The patient was a spoilt boy of 2 years who had been born and brought up in Nairobi. He had been in England 3 months. Three weeks before being seen he had been passing segments of tapeworm in the stools. One of the proglottides was sent to the Maidstone laboratory where the pathologist, with great acumen, reported that it was a segment of *Inermicapsifer cubensis*. As it is not possible to make the diagnosis without examination of the scolex it is difficult to guess how this identification was arrived at.

The child was apparently very healthy and the eosinophilia in the blood 5 per cent. As he was a difficult child it was decided to give him extract of filix mas, min 15, in jam at 6.30 p.m. He had syrup of figs early next morning and without any particular preparation produced two dead cestodes each 25 cm. in length. In one the head had been damaged, in the other it was perfect.

Very little information could be obtained about the possible source of infection. The child had not been entrusted to native servants for long and the two nurses he had had been Seychellois. On two occasions he had been on picnics in the neighbourhood of Nairobi, and it is possible that he had swallowed some insect which is the normal intermediary of *Inermicapsifer*.

I. arvicanthidis is normally a parasite of various kinds of rats in Africa. A number of other species of *Inermicapsifer* also occur in African rodents and

Hyaconidae. Their intermediate hosts are not known, but are probably small insects or other invertebrates which might be accidentally swallowed by man.

A species which has been called *Iaerwacapsifer caberius* (Kouri, 1939), and has occurred several times in man in Cuba, may also be normally a parasite of some rodent or other animal, but this is not yet known.

Iaerwacapsifer is distinguished from *Rastilleus* by the absence of hooks in the suckers.

Mr P G Shute

The comparative distribution of oöcysts of human malaria parasites on the stomach wall of *Anopheles*

1 *Anopheles (Myzomyia) stephensi*.

2 *Anopheles (Anopheles) maculipennis* var. *atroparvus*.

Infected specimens of *A. maculipennis* usually show oöcysts concentrated at the posterior end of the mid-gut. If however immediately after taking an infected blood meal the insect is stood on its head and kept in this position for 24 hours, the majority of the oöcysts are found at the anterior end of the mid-gut.

The secretion of the salivary glands of *A. maculipennis* has strong agglutinating properties so that following a blood meal the posterior half of the gut contains all the red cells and the anterior half the plasma.

Infected specimens of *A. stephensi* usually show oöcysts about equally distributed all over the gut, often with a few oöcysts at the extreme end of the anterior portion.

The salivary gland secretion of *A. stephensi* does not agglutinate human blood.

It is believed that *Anopheles* whose glands agglutinate blood will, as the result of this, cause the oöcysts to be situated at the posterior end of the mid gut, while in those species which do not cause agglutination, the oöcysts will be distributed more or less evenly all over the gut.

A number of specimens were shown.

Dr Thomas H White

Photographs of yaws lesions in Tanganyika Africans.

Eight photographs were shown, including a case of early gangosa in a child of about 10 years, who showed extensive scarring probably due to tertiary yaws. There was an ulcerating lesion of the nostrils with destruction of the alar cartilages. Other cases illustrated included curcinate and lichenoid lesions dactylitis, and yaws involving the scalp.

ORDINARY MEETING

of the Society held at

Manson House, 26, Portland Place, London, W 1

on

Thursday, 9th December, 1948, at 7 30 p m

THE PRESIDENT,

SIR PHILIP MANSON-BAHR, C M G , D S O , M D , F R C P ,
in the Chair

PAPER

A SURVEY OF PHYSIOLOGICAL STUDIES OF MENTAL AND PHYSICAL WORK IN HOT AND HUMID ENVIRONMENTS

BY

GUY P CROWDEN, O B E , T D , D S C , M R C P

Professor of Applied Physiology, London School of Hygiene and Tropical Medicine

It is with some temerity that I present this paper, for it is a departure from the usual type in that it is an attempt to appreciate the work of others rather than to present new or original matter

In the field covered by the title of this paper early experimenters, in spite of their meagre facilities for research, have made notable contributions to knowledge and asked questions which have as yet been only partially answered. In recent years, during times of war emergency, intensive researches on human reactions to heat and humidity have been carried out to help man face climatic extremes not only in theatres of war but in the engines of war and in industry engaged on vital production. It is becoming more and more recognized that there is indeed urgent need for the application of scientific knowledge to human problems arising out of man's physiological and psychological limitations.

In the interests of the positive health of present and future generations, it is incumbent on us to try and ensure that knowledge gained is so interpreted and made available that its use, if use it has, may contribute to human welfare in times of peace. Frankly, it is with that object in view that this paper has been prepared, and although I am only too conscious of its inadequacy I hope that it may still be of some service.

FORDYCE AND BLAGDEN (1774).

In order to view the physiological studies of our own time in some perspective it is of particular interest to recall the pioneering experiments of two medical men GEORGE FORDYCE and CHARLES BLAGDEN who, as long ago as 1774 subjected themselves and a team of their friends to really astonishing degrees of heat and humidity. Usually their spectacular observation that a beef steak was cooked by the hot air in the room while they were present, is the only reference to their work. On consulting the original records, it is surprising to find how far in advance of their time were their physiological observations. GEORGE FORDYCE, M.D. (Edin.) was a physician at St. Thomas's Hospital from 1770-1802, and his friend CHARLES BLAGDEN also M.D. (Edin.), was a medical officer in the Army till 1814 and Secretary of the Royal Society in 1784. Fortunately BLAGDEN recorded details of these early experiments in two papers which were published in the Philosophical Transactions of the Royal Society in 1775 under the title "Experiments and Observations in an Heated Room."

FORDYCE had three hot rooms prepared for the experiments. In one the air temperature ranged from 110-120° F. coupled with a high degree of humidity obtained by pouring boiling water on the floor—a procedure which necessitated the wearing of wooden clogs. The following is a quotation from BLAGDEN's first paper in which he recorded his friend's reactions:

"He then entered the first room, and stood in the parts heated to 110°; in about half a minute his shirt became so wet that he was obliged to throw it aside and then the water poured down in streams over his whole body. His viog remained 10 minutes in this heat of 110° he removed to the part of the room heated to 120° and after staying there 20 minutes, he found that the thermometer placed under his tongue and held in his hand, stood just at 100° and that his urine was of the same temperature. His pulse had gradually risen till it made 145 pulsations in a minute. The external circulation was greatly increased, the veins had become very large and an universal redness had diffused itself over the body attended with a strong feeling of heat."

It is evident from this record that they recognized increased peripheral circulation, increased pulse rate and increased body temperature as physiological reactions to heat. In another experiment the air temperature was raised to 130° F. and it was noted that moisture condensed on the surface of a flask containing water at 100° F. from which I calculate that the wet bulb temperature was at least 105° F. BLAGDEN placed it on record that higher air temperatures could be endured when the air was dry than when it was wet, a most interesting observation which, more than 120 years later, was supported by the researches of the late Professor J. S. HALDANE and his field observations in mines and ships of the Royal Navy and more recently still by the intensive work in 1944 of ESCIMA and his colleagues working at the Armoured Medical Research Laboratory at Fort Knox in the United States.

Before attempting to deal with the more exact studies of recent times, one further reference must be made to FORDYCE and BLAGDEN's experiments, namely to those on dry heat at temperatures as high as 260° F. They took the

precautions of heating up the room the day before to ensure that the walls were thoroughly warmed, a precaution which is now current practice in such research. They placed on record the observation that water exposed in a vessel in the room rose to 140°F in 1 hour, while in another vessel with the water covered with a film of wax the temperature rose to 152°F in the same time and that the water finally boiled. From this physical observation and their own personal reactions they were led to make the following comment on the role of sweating in body temperature control

"Perhaps no experiments hitherto made furnish more remarkable instances of the cooling effect of evaporation than these last facts, a power which appears to be much greater than hath commonly been suspected. The evaporation itself was more considerable in our experiments than it can be in almost any other situation, because the air applied to the evaporating surface was uncommonly hot, and at the same time not more charged with water than in its ordinary state. A powerful assistant evaporation must undoubtedly prove, in keeping the living body properly cool, when exposed to great heats, but it can act only in a gross way, and by no means in such a nice proportion to the momentary exigencies of the animal as would be requisite for the exact preservation of its temperature that other provision of nature which seems more immediately connected with the powers of life, is probably the great agent in preserving the just balance of temperature exerting a greater effect in proportion as the evaporation is deficient, and a less effect as evaporation increases."

It should be noted that FORDYCE and BLAGDEN and their friends did not carry out muscular work in their heated rooms. Had they done so they would have been less confident than they were led to be by their limited observations as to the human body's capacity to withstand great heats. In 1876, CLAUDE BERNARD in his work on "Animal Heat and the Effects of Heat" supported their view that moist heat was more rapidly harmful than dry heat, but he could not agree with them in regard to the capacity of the body to maintain its temperature when exposed to very high external temperatures.

The following is the reference in BLAGDEN's paper to the beefsteak test for room temperature which, incidentally, they carried out to prove to critics that the air was really hot

"In about 20 minutes the eggs were taken out roasted quite hard, and in 47 minutes the steak was not only dressed but almost dry. Another beef-steak was rather over-done in 33 minutes. In the evening, when the heat was still greater, we laid a third beef-steak in the same place and as it had now been observed that the effect of heated air was much increased by putting it in motion, we blew upon the steak with a pair of bellows, which produced a visible change on its surface, and seemed to hasten the dressing, the greater part of it was found pretty well done in 13 minutes."

FORDYCE and BLAGDEN had no wet bulb thermometer to check the humidity of the air. They lamented the fact that they had no balance with which to estimate sweat loss. They had no means of controlling the heat and humidity of the air in their experimental rooms at any desired level, an essential requirement for accurate work and one which could not be adequately met until more than 130 years later success in this direction attended the pioneering work of W. H. CARRIER in the United States in air conditioning engineering.

J. S. HALDANE (1905).

In reviewing studies of this kind it is of particular value to follow the lines of thought stimulated by records of difficulties encountered and questions asked and to ascertain if and when and particularly how such difficulties or questions have been met or answered.

In the present survey it is logical to proceed at once to consider the work of the late Professor J. S. HALDANE, whose paper on "The Influence of High Air Temperatures" (1905) is an important milestone in progress towards a true understanding of the physiological reactions of man to the thermal characteristics of his external environment. In more senses than one HALDANE's work was notable. His interest in the subject had been aroused when his attention was drawn to the human problems of work under conditions of heat and humidity met with in the Cornish tin mines and the cotton and flax textile industries. He coupled his field observations with experiments on himself, his colleague, C. G. DOOLAN, and the late Professor A. E. BOYCE in the warm incubator room at the Lister Institute, in a heated room in the Physiological Laboratory Oxford, and in a Turkish bath. His facilities for such work were by no means ideal, but with improved instruments and technique he recorded the physical characteristics of the environment with greater accuracy than had been possible in earlier experiments not only in respect of wet and dry bulb temperatures but also of air movement. He studied physiological reactions at rest and when performing muscular work. From his observations of change in rectal temperature, pulse rate sweating and records of the subjective sensations and signs of physiological stress under conditions of heat and humidity ranging from 70° to 108° F dry bulb and 61° to 88° F wet bulb he was led to the conclusion that

It is clear that in still and warm air what matters to the persons present is neither the temperature of the air nor its relative saturation, nor the absolute percentage of aqueous vapour present, but the temperature shown by the wet bulb thermometer. If this exceeds certain point (about 76° F or 25.5° C.) continuous hard work becomes impracticable and beyond about 88° F or 31° C. it becomes impracticable for ordinary persons even to stay for long periods in such air although practice may increase to some extent the limit which can be tolerated. In moving air on the other hand, the limit is extended by several degrees.

It should be noted that HALDANE had in mind ordinary persons, not acclimatized young men in vigorous health.

HALDANE's recognition of the physiological significance of the wet bulb at high air temperatures was a most important advance and one which has stood the test of time but we must not overlook the fact that it does not take into account all the physical factors concerned either in determining thermal comfort sensations or the endurable or desirable thermal conditions for work. For example FORDYCE and BLADEN had noticed that at very high dry bulb temperatures air movement aggravated discomfort, and HALDANE recorded the observation that at about 100° F dry bulb an air current of 173 feet per minute enabled a wet bulb temperature of 83° F to be borne without abnormal rise of body temperature.

Then there is the radiant heat factor to be taken into account, particularly in certain industries and climates. Thus, in 1913, E H HUNT, in his paper on "The Regulation of Body Temperature in Extremes of Dry Heat," commented on the amount of water consumed by men walking for long periods in the sun in India. In trying to equate total metabolism and heat loss by the evaporation of sweat, he lamented the fact that he had no data from which to estimate the heat added to the body by direct solar radiation. In this latter connection it is of interest to note that H F BLUM (1945), of the U S Naval Medical Research Institute, Maryland, in a paper on the "Physiological Effects of Sunlight on Man," gives an approximate estimate of the total solar heat load under desert conditions as about 240 kilocalories per hour. He pointed out that this figure is approximately equal to the metabolic heat load of ordinary walking at 3 miles per hour, and concludes that the solar heat load cannot therefore be disregarded when considering the total heat load under such conditions. In his calculations, BLUM (1945) made use of the figure of 57 per cent absorption of solar radiation by average blonde skin and khaki cloth given by Sir CHARLES J MARTIN in 1930 in his Croonian Lecture on "The Thermal Adjustment of Man and Animals to External Conditions."

1914-39

During the war years 1914-18 attention became focused on problems of the human factor in industry owing to the vital necessity for maintaining maximum production in munition factories. The Health of Munition Workers' Committee, and later the Industrial Fatigue Research Board of the Medical Research Council, instituted investigations in which the thermal environment was studied in relation to output and accidents. In one investigation by H M VERNON (1919) it was clearly demonstrated that the provision of good ventilation, particularly during the summer months, was essential for the maintenance of production in hot and heavy work in tinsplate factories. The katathermometer introduced by Sir LEONARD HILL in 1914 was later used in many investigations in industry and the rate of cooling of that instrument or "cooling power" of the air, as well as the dry bulb and wet bulb temperature of the air, were related to thermal comfort sensations, efficiency of performance, output and the incidence of accidents. These enquiries yielded a great deal of evidence that all was not well in industry from the physiological point of view, and more important still they indicated the directions in which the improvement of working conditions should take place.

At this stage it is pertinent to draw attention to the fact that the progress of physiological research on the effects of hot and humid environments has largely depended on progress in other fields, notably in air conditioning engineering and in the development of scientific instruments for the ready and accurate assessment of the physical characteristics of the environment, namely, temperature, humidity, air movement and radiant heat.

The development of dew-point control in air conditioning practice by W. H. CAHNER, of the American Society of Heating and Ventilating Engineers materially facilitated physiological research, for it enabled any desired dry bulb or wet bulb temperature to be maintained in experimental rooms. In 1923 two engineers F. C. HOUGHTON and C. P. YAGLOU published details of a very intensive experimental research on thermal comfort carried out in two adjoining air conditioned rooms at the Research Laboratory of the U. S. Bureau of Mines at Pittsburgh. In these experiments the air conditions in one room were varied until subjects passing into it from the other judged that they experienced no change in thermal sensation. In this way it was shown that various combinations of dry bulb, wet bulb temperatures and air movement produced the same sensation as that experienced in still, saturated air at a definite temperature named by them as the Effective Temperature. In this way the Effective Temperature Scale was built up.

The Effective Temperature Scale thus enables various combinations of dry bulb and wet bulb temperatures and air movement to be expressed in terms of a single index—the Effective Temperature, which is the temperature of still, saturated air in which the same thermal sensation of comfort or discomfort would be experienced. At the same time as experiments to determine the Effective Temperature Scale were taking place parallel experiments were carried out in collaboration with W. S. MCCONNELL, a surgeon in the U. S. Public Health Service, and it was shown that physiological reactions to the thermal environment, namely pulse rate, sweating and rectal temperature closely followed changes in Effective Temperature, a fact which has been confirmed by research in very recent times.

Two effective temperature scales were constructed, one for persons normally clothed according to American custom and the other called the "basic scale," for workers stripped to the waist.

Before passing on to other studies, I think it is of interest to refer to the procedure adopted by YAGLOU in some of his later experiments carried out in the air conditioned laboratory at the Harvard School of Public Health. In this connection it is worth while quoting some of the data from an experiment in which the physiological reactions of resting and working subjects were compared.

After a preliminary period of rest in an adjoining room, during which their pulse rates, temperatures and blood pressures were observed, the subjects entered the air conditioned room in which the air was saturated at 85 °F. The resting subject showed little reaction, and the working subject was easily able to stand up to the conditions although his temperature rose slightly and then remained steady while his pulse increased to about 100. In the second phase of this experiment the dry bulb was 137 °F and the wet bulb 84.3 °F. The resting subject showed a slow but gradual rise in body temperature and a pulse rate and an increased loss of weight due to sweating. The working

GUY P CROWDEN

subject's reactions were, however, markedly more pronounced than in the first phase, a fact I can testify to as I happened to be the subject YAGLOU called on me to stop pedalling the bicycle ergometer after half an hour as the pulse had risen to 160 and the rectal temperature to 102° F. The effective temperature in the second phase was 95 2° F in contrast to 85° F in the first phase. Only a few days ago I noticed that in that experiment carried out in 1929, the hot conditions in the second phase were very similar to those found by EICHNA and his colleagues in 1944 to be intolerable for muscular work of which he defined three ranges of temperature conditions which corresponded to the limits described by him as relatively easy, difficult and impossible.

The findings of that most interesting research by EICHNA and his colleagues are briefly summarized in Table I, which gives the averages for the group observations on physiological reactions such as pulse rate, rectal temperature, sweat loss and also the actual performance of the physical task.

TABLE I
HOT ENVIRONMENTS

Average values for group 13 men, aged 18 to 30, acclimatized, working stripped to waist

	Relatively easy (safe limits)	Difficult	Impossible
Dry bulb range	92.5-120° F	94-120° F	90-120° F
Wet bulb range	92.5-90° F	94-92° F	90-94° F
Effective temperature	91-94 4° F	92.8-95 0° F	95.3-97 2° F
Performance of work	100%	100%	Less than 50%
Pulse after 4 hours	Less than 130 per min	130-145 per min	Above 145 per min
Rectal temperature	Under 101° F	101-102° F	Above 102° F
Sweat loss per hour	1050-1687 c.c.	1241-1976 c.c.	1670-2689 c.c.
	Average 1433	Average 1560	Average 2289

(EICHNA *et al*)

Mental Work

I must now refer to studies of mental work under hot conditions. In this connection I particularly wish to draw attention to the valuable contributions to knowledge made by N. H. MACKWORTH, of the Medical Research Council Applied Psychology Unit, who carried out very detailed studies at the Psychology Laboratory, Cambridge, for the Royal Naval Personnel Research Committee during recent years.

At the Royal Society Empire Scientific Conference held at Oxford in 1946

MACKEWORTH communicated a paper entitled "Definition of the Upper Limit of Environmental Warmth by Psychological Tests of Human Performance. In his studies he used, as subjects, young, fit, acclimatized, naval personnel who worked stripped to the waist and performed tasks which involved mental and physical work or mental work only. He found that for men of this type there was a critical zone of effective temperature above which accuracy of performance of mental tasks declined. The zone as defined on the basis of his group observations, ranged from 83° F to 87.5° F effective temperature namely from 90° F dry bulb and 80° F wet bulb to 93° F dry bulb and 85° F wet bulb when the air movement is 100 feet per minute. In one series of tests in which accuracy in wireless telegraphy reception was measured, it was shown that the average incidence of faulty messages rose from approximately 15 per cent, at 79° F effective temperature to over 30 per cent, when the effective temperature was 92° F (Fig 1). In view of the importance of restful sleep for the maintenance of day to day efficiency experiments were devised so that restlessness during sleep could be automatically recorded. Nightly for a period of a month observations were made on six subjects, and it was shown that whereas they turned over heavily about 16 times during the night, when the effective temperature was 79° F., the number rose by 50 per cent to 24 movements per night when the effective temperature was 87.5° F (Fig 2). The significance of these studies to life and work in the tropics is apparent.

Recent Publications

The literature which falls within the scope of the title of this paper is indeed vast and it would be quite impossible in the limited time available to do justice for example to the work of C. E. A. WINDLOW and his colleagues, or H. C. BAXTER and others in U.S.A., or D. H. K. LEE in Australia, or D. BAXTER in this country. I cannot however conclude without naming certain recent publications by members of the best research teams which worked with E. A. CAUDMILLAN in the Medical Research Council's laboratories at the National Hospital, Queen Square and the field studies which arose out of their laboratory researches. The papers are as follows: Heat-Stroke and Heat Exhaustion in Iraq, by J. C. WATERLOW (1947). Effects on Man of High Temperatures, by W. S. S. LADLELL (1947a). Effects on Man of Restricted Water Supply, by W. S. S. LADLELL (1947b). Effects of Tropical Climate on Men in Warships, by F. P. ELLIS (1947). Biological Assessment of Clothing for Tropical Service Use, by J. S. WINDLER (1947). Desert Climate, by W. S. S. LADLELL, J. C. WATERLOW and M. PAULSEN. H. THOM (1944) and Problems of Naval Warfare under Climatic Extremes, by MACDONALD CARTERLEY (1945).

These papers deal primarily with physiological studies, but a very detailed examination of methods for assessing the physical environment was carried out at the same time. In this connection particular attention must be drawn to the publication entitled *Environmental Warmth and Its Measurement*, by T. BEDFORD (1946). This memorandum gives full details of methods for evaluating the thermal characteristics of the environment which are of physiological and psychological significance.

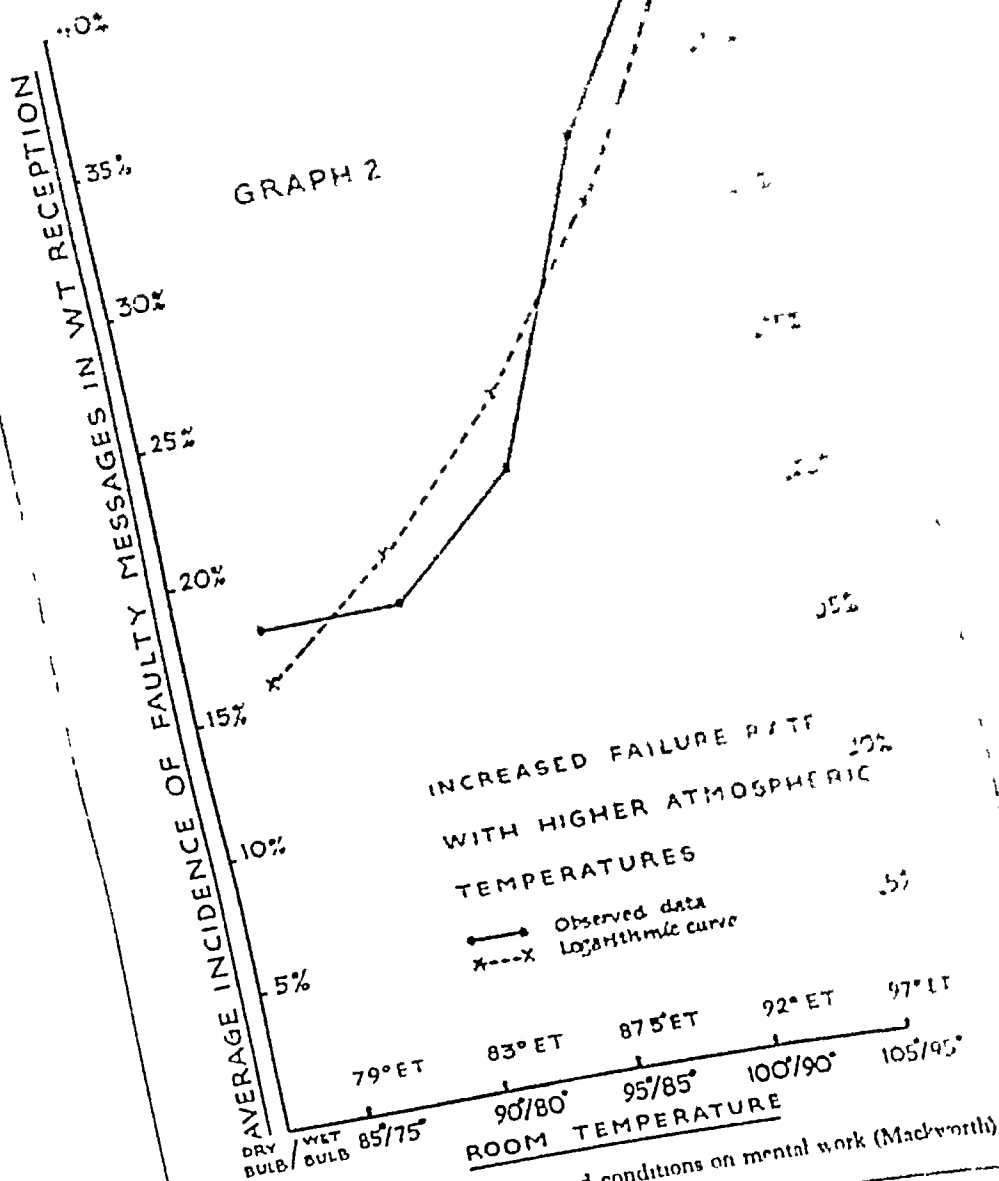


FIG 1—Effects of hot and humid conditions on mental work (Mackworth)

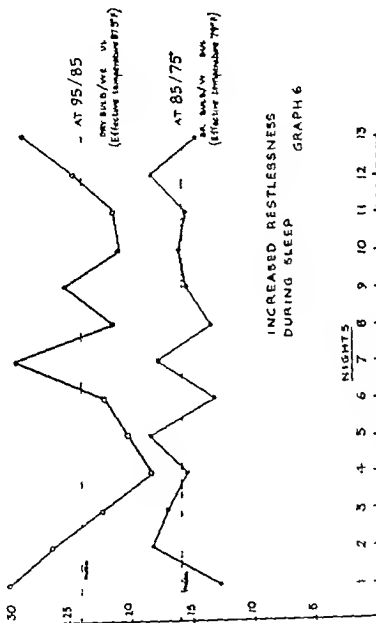


FIG. —Effects of hot and humid environment on restlessness of ring leop. (Blackworth.)

DISCUSSION

Dr J S Welner Professor CROWDEN in his survey has brought to the fore many of the more important problems which engaged the attention of the various climatic research teams during the war. Some of these are still under consideration in the Medical Research Council's Climatic and Working Efficiency Unit at Oxford. Unfortunately many of the more important results of the war-time Medical Research Council's Unit (Queen Square) have not yet been published, but it might be of interest to mention in particular the work which was done on the assessment of the physiological effects of high temperatures.

From the historical review that Professor CROWDEN has presented, and especially from his reference to the American effective temperature scale, it will be appreciated how necessary it is in practice to be able to give some combined assessment of the different factors making up the thermal load of working subjects at high environmental temperatures. These factors are the dry bulb temperature wet bulb temperature, air movement radiant heat, the clothing worn and the rate of work to mention the most important. The effective temperature scale has the drawback that no allowance can be made for radiant heat (although Dr BEDFORD has suggested in M.R.C. war memorandum No. 17 published by H.M.S.O., a tentative correction for this), nor does it allow for different intensities of work carried on, say over 3 or 4 hours. Moreover investigation in this country and in America has revealed that there are certain inadequacies in the scale as it stands. In particular it under-estimates the deleterious effects of low air speeds of the order of 30 feet per minute. The effective temperature scale as Professor CROWDEN explained, is one based primarily on subjective sensations, and hence for this reason alone extrapolations to high temperatures would be expected to be less reliable. On the basis of many experiments the Medical Research Council's War time Unit has proposed a combined assessment using as objective physiological index of heat load the output of sweat and the rise in body temperature, and it is believed that this new index should prove of greater applicability and reliability than effective temperature scale. In drawing up such a scale (or any other) it should be emphasized that acclimatization makes a very important difference to the working capacity and hence to the level of standards in terms of these indices of heat stress. The scale available at present refers to well-acclimatized European subjects. In applying such a scale and its standards to other situations the actual state of acclimatization must be taken into account.

The physiology of acclimatization has received a good deal of attention recently. The improvement in working ability which accompanies successive exposures to high environmental temperatures is often very striking. With the improvement, the body temperature, for a standard set of conditions, is adjusted to a somewhat lower level, and one finds therefore that the acclimatized man tends to sweat more easily and at the same rectal temperature, at a greater rate than the unacclimatized. The changes in circulation with acclimatization

are equally striking. For example, the pulse rate for a given amount of work becomes less with acclimatization. At Oxford we have given particular attention to the changes in the rate of blood flow in the periphery. These results we hope to publish in the near future. The unacclimatized individual is liable to heat syncope which is the well-known vaso-vagal syndrome, *i.e.*, is characterized by bradycardia and simultaneous peripheral vaso-dilatation. With acclimatization and reduction in general body temperature the liability to this disorder is greatly diminished. In recent years many other aspects of adaptation to high temperatures have been studied and only very few of these can be mentioned here. For example, much work in this country and America has dealt with the activity of the sweat glands and the factors influencing the composition of sweat. These results have a direct bearing on the water and salt requirements in the tropics and in the prevention of salt deficiency and dehydration in hot environments. The role of the kidney in conserving water and salt loss from the body has also been investigated. It has been found that the ability of the kidney to retain water and salt is probably independent of the dehydration and salt loss due to sweating and is a result of the heating up *per se*.

On the clinical side there have been studies of the syndromes of salt deficiency, tropical neurasthenia and heat stroke. I would call your attention to the very interesting studies of tropical anhidrosis by O'BRIEN in New Guinea (*Brit J of Dermatol and Syph*, 1947, 59, 121), WOLKIN in America and LADDELL and WATERLOW in Persia. In this syndrome the damage to and blocking up of sweat glands seems a reality. It has been regarded as an aftermath of prickly heat or been attributed to interference with sebaceous secretion. It may be that here we have the genesis of many cases of heat stroke.

This is a vast field of enquiry, and I am grateful for the opportunity you have given me to supplement from my personal experience the review that Professor CROWDEN has presented.

Sir George McRobert. It is to be hoped that the laboratory work of the opening speakers will quickly find practical application in the tropics. It is really extraordinary how the Englishman, especially of the official classes, has clung to the habits and habiliments of England, even in the hottest and stickiest climates. Worse still, he has tried to induce indigenous inhabitants to follow his example.

I look back with horror to the university convocations in Madras when, clad in a dark morning suit under red flannel doctor's robes, I painfully watched a plethoric and perspiring Chancellor, in the black and gold raiment of his counterpart at Oxford, conferring—at the hottest part of a moist and stifling August afternoon—individual degrees on a thousand or so new graduates, each wearing, according to regulations, a dark suit, black robes and a black mortar board.

That annual day of suffering made the university professor sympathize

with the Honourable Judges of His Majesty's High Court of Judicature who, clad in the judicial garments of the King's Bench Division in London sit 5 days a week, from 11 till 5 through the hottest tropical hours listening more or less patiently to Indian counsel pleading in the prescribed black gown and stiff bands. How dearly they earned their stipends!

For many years it has been usual for the governments of India and of its constituent provinces to move to summer capitals in the hills, in order better to devote their energies to higher policy and planning. This move which cost a vast sum annually was assailed by politicians and press alike as well as by European big business interests as unjustified and unjustifiable. Disputation was prolonged and vehement but no scientific evidence has ever been produced by either side. It is obviously a subject worthy of the serious attention of physiologists and psychologists.

Many words have been expended on the alleged need for violent physical exercise in the tropics. Most Europeans indulge in an orgy of exercise in the evenings and despise those who do not. Educationists encourage, nay drive their pupils to do the same. Is this wise? We have no reliable data.

How long does a European remain efficient in the tropics without home leave? How does local leave to the hills alter that period? Obviously the answer must vary with the tropical locality concerned, but up to the present time our recommendations have been made by guesswork. Physiologists and psychologists should be able to give accurate information after studying conditions on the spot. Stress has been laid on the desirability of sending to the tropics only those likely to acclimatize quickly. I should like to know how this acclimatization to heat and moisture so rapidly acquired by human guinea-pigs in the laboratories in this country is likely to last. Those of us with much tropical experience feel that the newcomer stands heat well in his first season, is not quite so efficient in his second, and that in the third and succeeding seasons there is a steady decline in efficiency but we have no scientific data to offer.

Despite the spread of self-government and independence to many tropical and subtropical lands I feel confident that for many years to come information vital to these young states can be obtained only by small well-equipped expeditions sent out by such institutions as the London School of Hygiene and Tropical Medicine. I feel sure that the greatest days of our English schools still lie ahead and that expeditions based on London and Liverpool have more than ever an important part to play in making the tropics habitable and healthy.

Dr O. B. Alakija. On the question of acclimatization mentioned by a previous speaker it was my experience in the tropics that whilst those of the more leisurely class are standing around and are literally covered in perspiration, labourers recruited from the indigenous people carrying quite heavy loads of upward of 112 lb have been known to work for an hour or so before commencing to sweat.

Referring to the occasions when the Governor or some high official is

DISCUSSION

present at some parade or other, as mentioned by another speaker, I have noticed that whilst those in the grand stands are sweating quite profusely, troops and police on parade standing in the sun and clad in heavy uniforms not always of a type suitable for the tropics, are mostly quite dry and appear quite cool. These troops are usually recruited from the labouring classes.

This brings me to ask whether this question of "acclimatization" to tropical heat is not connected with the general health and fitness of the individual, and I should be interested to hear Professor CROWDEN's opinion on the point.

Sir Henry Tidy Dr WEINER spoke of training and acclimatization. I have had some experience of training with athletes, and to a considerable extent one can measure fitness in training by the amount of sweating. When a man commences training he sweats a great deal more than he does later on. If he gets stale, which is a curious point that nobody can define, he sweats again. Obviously this is a different matter from acclimatization. One can have both training and acclimatization, but they are different points. I ask the question: What is the relation between training and acclimatization?

Lieut-Colonel J C Watts I feel that the apparent paradox raised by Sir HENRY TIDY is explained by the fact that in training we are increasing muscular efficiency and thus lowering heat production, whereas in acclimatization we are increasing our ability to lose heat. Obviously, heat production by the body and heat loss are intimately linked and we cannot consider one without the other.

Dr Weiner May I try and comment on the several questions on acclimatization which have been raised in the discussion. First of all as regards tropical people. The state of acclimatization of natives of the tropics has not yet come under exact study, that is to say standardized tests in standardized thermal conditions to enable direct comparisons with Europeans to be made have not yet been carried out. In the case of artificial acclimatization of Europeans one striking phenomenon is the fact that sweating is brought on much more easily and the actual output of sweat relative to the body temperature is rather greater. In the literature we have contradictory statements of opinion about the output of sweat by tropical peoples, but unless one really measures the output carefully, in fact, makes a complete thermo-dynamic balance sheet, it is hazardous to trust to casual observation. I have carried out one series of observations recently on Bantu mine labourers in Johannesburg. The results have not yet been published, but it does not appear that as regards the tropical and non-European after acclimatization in these conditions. Of course tropical people may very well be adapted to the hot conditions of tropical sunshine and no doubt the heavier skin pigmentation must be related to exposure to ultra-violet light. I must repeat again that until exact tests are done in the tropics we must suspend judgment on these questions.

Lastly the problem of what happens to Europeans after 2 or 3 years in the tropics is also very important. Any standards based on artificial conditions in hot rooms in temperate countries must be regarded only as tentative. It is even possible that the degree of acclimatization which men have obtained in hot rooms may in some circumstances be bigger than that ordinarily conferred by life in the tropics. The state of acclimatization depends on the intensity of work, length of exposure, and many other factors. The whole psychological and social situation is also very different in the tropics. Sunlight obviously alters the situation enormously from that of artificial hot rooms. The effect on the skin of ultra-violet light especially of sunburn, may perhaps exert a definite influence on the sweating mechanism.

Professor Crowden I am very pleased that Dr. WEINER has come up from Oxford to take part in this discussion. He came from South Africa some years ago to continue his researches on problems of heat and humidity on which he had been engaged with Dr. ORNSTEIN. The fact that throughout the war period he was intimately associated with the researches to which I have referred, makes his observations on the points raised in the discussion of particular value.

Dr. WEINER has already dealt with many of the questions raised by the speakers, but there are still some observations which I would like to make. The question of discomfort due to still air was raised. In this connection I made some observations at Lagos on the effect of mosquito nets on air movement. I found that in a hospital ward the air movement round a bed-patient was reduced from 80 to 10 feet per minute when the mosquito net was placed in position. This was equivalent to raising the effective temperature from 80° to 82° F. Such a change increases thermal discomfort considerably. It should also be noted that when under a mosquito net the thermal load on the body due to radiant heat from hot walls and ceilings is particularly unpleasant and causes increased sweating.

Sir GEORGE McROBERT and Dr. ALAKIJA have both raised the question of the effect of clothing. In this connection I would recall Sir CHARLES MARTIN's comment that the major handicap to the European in the tropics was his clothing. The native worker in the fields may wear a shirt and shorts, but he wears them in a different way. We wear our shirts inside our shorts not outside as he does. He makes full use of ventilation by so doing. Undoubtedly there is scope for further studies on clothing for the tropics, and also in industry at home and abroad.

I quite agree that there is urgent need for the findings of research to be applied, and that, following experiments in artificially heated rooms field test in the tropics must be carried out before specific practical recommendations are made.

Finally I would like to thank your Society for the opportunity of presenting this paper for consideration and the various speakers for their valuable contributions to the discussion.

COMMUNICATIONS

STUDIES ON A WEST AFRICAN STRAIN OF *PLASMODIUM FALCIPARUM*

THE EFFICACY OF PALUDRINE (PROGUANIL) AS A PROPHYLACTIC AGENT

BY

G COVELL, W D NICOL,
P G SHUTE AND M MARYON *

In the course of investigations carried out at Horton Hospital, Epsom, in 1930, attempts were made to infect *Anopheles maculipennis* var *atroparvus* bred from specimens collected in England, by feeding them on individuals harbouring gametocytes of an Indian strain of *Plasmodium falciparum*. None of the insects became infected, although the same species of mosquito was found readily susceptible to infections with strains of *P falciparum* originating in the Roman Campagna and in Sardinia (JAMES, 1931, JAMES, NICOL and SHUTE, 1932) † Attempts to infect *atroparvus* with two African strains, one from West Africa, the other from East Africa, were also unsuccessful, but in neither case was there a high density of gametocytes, so that there remained some doubt as to whether *atroparvus* was entirely refractory. Some years later an opportunity arose of repeating these observations with another West African strain of *P falciparum*, and in this case gametocytes were plentiful in the peripheral blood of the patient, there were numerous ex-flagellating males and ookinetes were observed in the stomach contents of the mosquitoes which had been allowed to feed on him. No oocysts, however, were seen on the stomach wall of any of the insects dissected (SHUTE, 1940).

In October, 1947, two of us (P G S and M M) visited Nigeria, under the auspices of the Medical Research Council, with the object of undertaking further studies on the malaria parasites of West Africa, taking with them

* We wish to acknowledge the assistance of Professor N HAMILTON FAIRLEY, FRS, in planning this experiment, and of Mr R J BROMFIELD, of the London School of Hygiene and Tropical Medicine for determining the concentration of paludrine in the blood of the subjects of the trials.

Since going to Press a paper by COVELL, G, NICOL, W D, SHUTE, P G and MARYON, M has been published in the *British Medical Journal* (15th January, 1949). This embodies the same experimental data and conclusions which are set out here and, in addition, gives details of the treatment, by paludrine and other drugs, of malaria caused by this West African strain of *Plasmodium falciparum*.

† A strain of *P falciparum* originating in Roumania was subsequently maintained for several years at Horton and this also was readily transmitted by the English *atroparvus*.

approximately 3 000 female *anopheles*. The journey which occupied 21 hours was made by air and the great majority of the insects survived. No gametocyte carriers were available on arrival, but some days later three of the local African children were found to have crescents in their peripheral blood. The surviving mosquitoes were allowed to feed on these, and some 200 of those which took a blood meal were subsequently dissected, but none was found to be infected, although oöcysts were observed on the stomach wall of several specimens of *A. gambiae* fed at the same time on the same individuals.

Defibrinated blood from an African child naturally infected with *P. falciparum* was brought back from Lagos to England and inoculated into a patient in Horton Hospital awaiting malaria therapy who had no previous history of malarial infection. The Lagos strain of *P. falciparum* was maintained at Horton by successive blood inoculation until February 1948. Meanwhile an insectary colony of *A. stephensi* (Type form) had been established at the Ministry of Health's Malaria Laboratory Horton, from specimens imported by air from Delhi, India. This species of *Anopheles*, which is a well known malaria vector in India, Iraq and the Persian Gulf proved to be highly susceptible to infection with the Lagos strain of *P. falciparum*, and it has been used for transmission in the investigations here reported.

DETAILS OF PROPHYLACTIC TRIALS

The investigation was commenced on the 17th May 1948. The subjects of the trial were patients in Horton Hospital, all of whom had undergone malaria therapy for neurosyphilis with the Madagascar strain of *P. vivax* or with European strains of *P. falciparum* 9 or more years previously but had not been exposed to malarial infection in the intervening period. They were arranged in groups as shown in Table I.

TABLE I

Group	Number of each group	Drug administered	Dosage
I	5	Paludrine	100 daily
II	5		50
III	5		100 twice weekly
IV	5		300 once
V	$\begin{cases} 1 \\ 1 \end{cases}$	Quinine dihydrochloride	Group: 8 daily 10
VI (control)	6	No drug	

Infection was given once weekly over a period of 6 weeks, each subject being bitten by five to ten heavily infected mosquitoes or receiving by intra

Except in the case of the two individuals under quinine prophylaxis (Group V) who were infected three times over a period of 3 weeks.

venous inoculation a suspension of the salivary glands of one heavily infected mosquito by the technique described by SHUTE (1937), alternately. After the first fortnight, infections were given on a different day each week, so as to simulate as closely as possible conditions which might arise in nature. A card was made out for each individual under drug prophylaxis, and on it was entered a record of every dose of the drug administered, each entry bearing the signature of two members of the nursing staff. On most occasions a medical officer was also present when the drug was given. Drug administration commenced 3 days prior to the first infection in each group, and was continued until 6 days after the last infection. Blood examinations (thick smears) were made twice weekly as a routine, and daily in the case of any rise of temperature above the normal level.

RESULTS

Group I One member of this group had a rise of temperature to 100° F on one day only, 14 days after his third infection and 4 days after his fourth.* No parasites were seen in the peripheral blood at any time. None of the other four members of the group showed pyrexia or parasites at any time.

Group II One member of this group had intermittent pyrexia for 4 days, commencing 15 days after his first infection (mosquito bites), 8 days after his second (sporozoites i.v.) and 2 days after his third (mosquito bites), the highest temperature recorded being 102° F. No parasites were seen at any time in the peripheral blood, and subinoculation of 20 c.c. blood intramuscularly into a non-immune subject proved negative. The donor was found to have a very low (practically nil) concentration of paludrine in his blood when this was examined during the pyrexial period, whereas a few days later the concentration was of the same order as that of the other four members of the group. The inference drawn was that he had in some way avoided taking the drug regularly as prescribed. That this could occur despite the rigorous system of inspection under which the trials were conducted illustrates the extreme difficulty of ensuring the regular administration of a prophylactic drug, even among persons under more or less strict disciplinary control. None of the other four members of the group showed pyrexia or parasites at any time.

Group III One member of this group had a rise of temperature to 99° and 100° F on the 10th and 11th days respectively after his second infection.* Another had a rise of temperature to 99.5° F 16 days after his second and 5 days after his third infection.* No parasites were seen in the blood of either of these two cases, and the remaining three members of the group showed neither pyrexia nor parasites at any time.

* The possibility that these slight rises of temperature were due to a modified malarial infection cannot entirely be ruled out, but it should be noted that similar "spikes" are seen from time to time in the temperature records of neurosyphilitic patients in the absence of malarial infection. A recent record of the temperatures of 21 such patients in Horton Hospital showed three such "spikes" over a period of 6 weeks.

Group II No member of this group showed pyrexia or parasites at any time.

Group I Case 1 (receiving quinine hydrochloride grain 5 daily) showed neither pyrexia nor parasites whilst taking the drug. A febrile attack, with demonstrable parasites in the peripheral blood, commenced on the 15th day after the last infection, 7 days after the drug was discontinued. Case 2 (receiving quinine hydrochloride grain 10 daily), likewise showed neither pyrexia nor parasites during the period of drug administration. A febrile attack, with demonstrable parasites in the peripheral blood, commenced 20 days after the last infection and 12 days after the drug had been discontinued.

Group II (Controls). All five members of this group developed overt attacks of malaria, with demonstrable parasites in the peripheral blood within the normal incubation period (7 to 14 days), thus demonstrating that each batch of mosquitoes used was infective (Table II).

TABLE II.

Case number	Date infectal.	Length of incubation period.	Length of pre-patent period.
1	May 22	10 day	8 day
2	June 1	12	14
3	8	12	14
4	17	7	
5	24	13	13

Ten weeks after the cessation of drug administration, during which time none of the 20 individuals who had received paludrine throughout the period of infection showed any rise of temperature above normal or demonstrable parasites in the peripheral blood, 18 of them were inoculated intravenously with sporozoites of the same strain of *P. falciparum* for the purpose of therapeutic trials. All without exception developed overt attacks of malaria with demonstrable parasites in the peripheral blood within a period of 7 to 10 days, thus proving their susceptibility to the Lagos strain.

Three members of the laboratory staff were bitten repeatedly whilst handling heavily infected batches of *A. stephensi* throughout the period of the investigation, taking no precautions to avoid infection. Each took paludrine mg. 200 in a single dose twice weekly at 3 to 4 days interval. None of them developed malarial attacks.

It is of interest also that the two members of the staff who visited Nigeria were inoculated intravenously in Lagos with a suspension of the salivary glands of a heavily infected specimen of *A. gambiae* caught in nature. The species

of parasite cannot be stated with certainty, but since the great majority of the positive blood smears examined at this time contained parasites of *P. falciparum*, it is more than likely that they were infected with sporozoites of this species. They had taken paludrine mg 100 daily until the day prior to infection inclusive, after which the drug was discontinued. Neither developed signs or symptoms of malaria.

COMMENT

The numbers of individuals in the groups treated were small, but the infections were probably a good deal heavier than those likely to be incurred by persons residing in malarious countries under natural conditions, particularly in respect of the intravenous inoculation of sporozoites, so that the prophylactic action of the drugs was submitted to a test of considerable severity.

There was no definite break through under any of the four paludrine regimes under trial. The only individual whose temperature record was suggestive of a malarial attack was the member of Group II (dosage mg 50 daily), who experienced a rise of temperature each evening over a period of 4 days. As noted above, no parasites were seen at any time in the peripheral blood, and subinoculation proved negative. The extremely low concentration of paludrine in the blood found at the first examination renders it almost certain that the drug had not been taken regularly as prescribed. Whilst, therefore, it is probable that the pyrexia was the result of malarial infection, this case cannot be classed as a genuine break through.

The fact that the two cases on quinine prophylaxis developed overt attacks of malaria shortly after ceasing to take the drug, whilst none of those who had been treated with paludrine during the period of infection did so, emphasizes the contention of FAIRLEY *et al* (1946) and other observers that paludrine acts as a true causal prophylactic in *P. falciparum* infections, whereas the effect of quinine is suppressive only.

Whilst all the four paludrine regimes used in these trials appeared to be adequate, we would confine our recommendations as regards West African strains of malaria to two only, namely (i) a daily dose of paludrine mg 100* for non-immune individuals such as European military personnel or civilians residing in malarious areas, and (ii) a single weekly dose of paludrine mg 300 for semi-immune persons such as native labour forces or Government employees recruited locally, the drug being administered wherever possible on pay day in the presence of the disbursing officer. We do not favour the twice weekly regime of paludrine mg 100, because this leaves an insufficient margin of safety, and moreover the drug is practically never taken on such a regime with the meticulous regularity necessary for adequate protection. We regard also a

* Children of 10 years and over may be given the same dosage as for adults. Those aged from 5 to 10 years may be given mg 0.50 daily, and those below 5 years of age mg 0.25 daily.

daily dose of paludrine mg. 50 only as offering an insufficient margin of safety bearing in mind the risk of an occasional omission to take the prescribed dose.

SUMMARY

1 The investigations recorded relate to a strain of *P. falciparum* obtained in November 1947 from a native of Lagos, Nigeria, West Africa.

2 Twenty non immune individuals (four groups of five in each group) were subjected to repeated infections with this strain of parasite over a period of 6 weeks. During this time Group I received paludrine mg. 100 daily. Group II mg. 50 daily. Group III mg. 100 twice weekly and Group IV mg. 300 once weekly. None of the members of these four groups developed an overt malarial attack during the period of drug administration or subsequently.

3 Two persons taking respectively quinine hydrochloride grain 5 and grain 10 daily were infected in a similar manner as those in Groups I to IV. The infection was effectively suppressed in both cases whilst the drug was being taken, but both developed overt attacks of malaria 7 and 12 days respectively after it was discontinued.

4 Although paludrine acted as a true causal prophylactic of the Lagos strain of *P. falciparum* in each of the regimes under trial it is considered that non-immune persons residing in or visiting West Africa should be advised to take not less than mg. 100 of the drug daily for the prophylaxis of malaria. For semi-immune subjects such as native labour forces and locally recruited Government employees a weekly dose of paludrine mg. 300 is recommended.

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A YAWS CAMPAIGN IN SIERRA LEONE

BY

R D HARDING, D.M., M.R.C.P., D.T.M. & H.,*

Formerly in charge Yaws and Sleeping Sickness Campaign, Sierra Leone

This paper is intended not so much as a scientific contribution to knowledge of yaws and its treatment, as a practical account of the methods which have been adopted in Sierra Leone in an attempt to control the disease in certain areas, the broad results achieved, and the failures and difficulties that have been encountered.

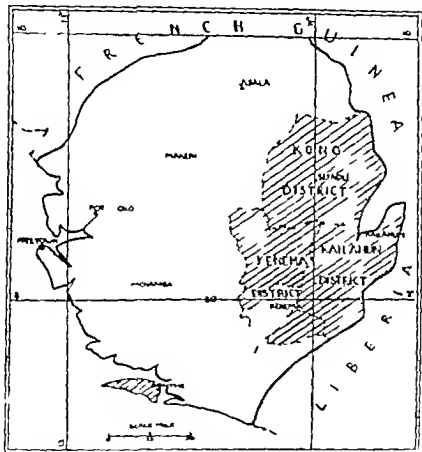
The only published accounts of large-scale yaws campaigns conducted on anything like scientific lines which have come to the writer's notice are the classical reports of the Jamaica Yaws Commission (1933-1936), VAN NITSSEN'S (1944) account of the campaign in the Belgian Congo, and LAMBERT'S description of a campaign in Samoa (LAMBERT, 1936). As STANUS (1939) remarks "Anti-yaws campaigns have been launched on a very large scale, but unfortunately, in most cases, no proper survey of the problem in hand has been made, no effective control has been established, and the results of the particular therapeutic measure have not been adequately followed, so that no very definite conclusion can be drawn as to the efficiency of these attempts at mass treatment."

The area dealt with in this paper comprises a block of country in the east of Sierra Leone lying roughly between latitudes $7^{\circ} 30'$ and 9° N, and longitudes $10^{\circ} 20'$ and $11^{\circ} 20'$ W, containing Kono, Kailahun, and part of Kenema Districts. The population of this area is approximately 276,000, and practically the whole population has been examined at least once, and all active yaws cases found at the time of examination have been treated, but in only perhaps one-third of the area have permanent treatment centres for continuing control as yet been set up (i.e., by the end of 1946). About 58,000 cases of yaws have been treated by teams and centres up to this time. More than

* Acknowledgment is due to Drs. APTE, HUTCHINSON, LUKE, MICHIE, PEASTON and RUSHTON, of the Colonial Medical Service, who, when attached to the Yaws Campaign, collected some of the data quoted, to Dr. MINNIE GOSDEN, Senior Pathologist, Sierra Leone, for carrying out the Kahn tests, and to Dr. W. P. H. LIGHTBODY, C.B.E., Director of Medical Services, Sierra Leone, for permission to publish this paper. I also wish to thank Dr. C. J. HACKETT, Director of the Wellcome Museum of Medical Science, for helpful criticism and advice.

half the population is of the Mendi tribe, the remainder consisting of the Kono and Kisi tribes. The great majority of the people are primitive peasants who live in insanitary mud huts and walk unshod, and the children go naked or very scantily clothed.

In the area concerned the vegetation consists in the southern half largely of secondary evergreen bush resulting from dense regrowth following cultiva-



tion, though some primary forest persists in patches. In a smaller zone in the north and north-east there is little evergreen bush, which is replaced by elephant grass except for the fringing forest—fine quite dense, along streams. In the intermediate zone the vegetation is of transitional type—areas of secondary thicket alternating with patches of elephant grass. The underlying rock is igneous. The country is in general undulating but intersected by mountains to the north and north-east. Much of the land is not well drained and swamps

are numerous. Laterite comes to the surface in places, and in the mountainous regions bare rock or rock covered with a thin layer of soil occurs, but over the greater part of the area and in all the valleys there is a fertile clayey top soil. Apart from individual mountains the altitude rises from some 400 feet in the south-west to nearly 2,000 feet in the north-east.

The annual rainfall averages some 90 to 95 inches. The period of heavy rainfall lasts from June to October, but storms commence in March during which some 3 inches usually falls, and there are heavier storms in April and November which brings the figure for each of these 2 months to 5 inches or more. Thus there is a long wet season during which humidity is high. December to February are nearly dry and during the day relative humidity is low, but dew falls on nearly every night of the year. The mean annual temperature is about 75° F, and the mean annual relative humidity about 83 per cent.

Yaws is found all over the area but differs considerably in prevalence. It has not been found possible to correlate the distribution with the vegetation, soil, or altitude, though there is undoubtedly a correlation with social status and with hygiene, and the more primitive Kissis have a higher incidence of yaws than the other tribes. A difficulty arises in comparing the yaws incidence in different areas in that great seasonal variations in the prevalence of active yaws occur in the same locality. Efflorescence of most types of secondary lesions occurs during the rains but they tend to regress or disappear in the dry season, so that surveys carried out at different times of the year are not comparable. Again the local distribution of the disease has been influenced by the presence of the three hospitals, one Government and two Mission, which exist in the area, as mentioned below. The only region where the yaws incidence is naturally lower, apart from the influence of tribe or hospitals, is in the south-east corner where, surprisingly enough, vegetation is particularly dense and there is a good deal of primary forest on the hills. In the drier elephant grass country beyond the northern boundary of the area under consideration there is also less yaws to be found.

Since the seasonal variation in the prevalence of yaws lesions is so important in Sierra Leone, and will be referred to again, it is desirable at this point to quote some figures obtained in the course of a special study designed to elucidate this factor. In this study monthly examinations were carried out over a period of a year of the individuals comprising a community of 1,400 persons in which yaws was endemic, and at each examination the frequency of different types of yaws lesions was noted. Treatment was withheld during the period except in a few cases insufficient in number to affect the general picture. The periodic frequencies of two types of lesion are shown in Table I. It will be seen that the incidence of infectious yaws rises from a minimum at the end of the dry season to a maximum towards the end of the rains.

The proportion of the population which has been infected with yaws at some time or other since birth is not known in most parts, but a questionnaire in a community of 1,300 people of the Kissi tribe showed that about 70 per cent had been infected, and in a Kono community where yaws was not so prevalent,

37 per cent. of 467 people questioned gave a history of infection. On the average in the whole area probably about two-thirds of people aged 10 or over have had yaws—the rate in children born since the yaws campaign started is no doubt falling in some regions.

TABLE I.

SEASONAL VARIATION IN THE PERCENTAGE INCIDENCE OF (a) INFECTIOUS YAWS LESIONS AND (b) ACTIVE YAWS IN CTION PLANTAR Y (c) MARKED LESION ACCOMPANIED BY (d) EXTENDED CONJUNCTIVITIS OF 1 600 PERSONS.

Season	Wet.	Dry.	Wet.			
Month	Oct. Nov.	Dec. Jan.	Feb. Mar.	Apr. May	June July	Aug. Sept.
(a) Inf. lesions	4.4	1	1	2.2	1	4.6
(b) Active lesions	16.4	11.9	8.9		4	11.6

The incidence of yaws lesions at the time of any one examination is of course generally very much less than these figures. The incidence at first examination in a few chiefdoms taken as a representative sample is given in Table II.

TABLE II.

CHIEFDOM, TRIBE, POPULATION, PERCENTAGE INFECTIOUS YAWS LESIONS, PERCENTAGE NON-INFECTIOUS YAWS LESIONS, TOTAL PERCENTAGE OF EXAMINATIONS.

Chiefdom	Tribe	Population	Infectious as Per cent	Non-infectious as Per cent	Total as Per cent	Season of examination
Kono Tege	Kono	4 500	4.4	3.9		Dry
Mafindu	Kono & Kono	3 600	13	4.2	24	Wet
Kamara	Kono	3 900	3.3		1.1	
Nani Koro		114		4.3	7.1	Dry
Lepidaria	Mende	4 000		40	79.1	Wet
Dodo		400	3.4	1.7	1	
Jalubian		16 000		3	3	Dry

Mafindu chiefdom is remote from any hospital or dispensary so that comparatively few of its inhabitants had received previous treatment. In both Nani Koro and Jalubian chiefdoms mission hospitals exist and have treated yaws cases seeking treatment for many years past. Most patients attend for from only one to three injections and a greater proportion of infectious than of non-infectious cases seek treatment, so that the effect (such as it is) over many years is to keep down the incidence of infectious yaws to low

level in their vicinity, while exerting less influence on the incidence of non-infectious yaws. The season at which examination was carried out has also influenced the figures.

As here classified, infectious yaws consists of primary and secondary lesions in which spirochaetes may be expected to exist on the surface, and comprise primary and secondary framboesiomata, condylomata, and pseudo-granulomatous eruptions of the soles (crab yaws). All tertiary lesions, including open lesions such as ulcers and gangosa, are classed as non-infectious, as also are the large variety of dry secondary lesions such as papular, squamous, hyperkeratotic, ringworm-like, etc., lesions of the soft skin, secondary bone and joint affections, and all non-granulomatous lesions of the palms and soles, such as hyperkeratosis, clawing, erosions, cracks and fissures, etc. Infectious lesions of course may, and often do, give way to non-infectious ones, and conversely, infectious papules may develop on top of non-infectious secondary lesions. Though HACKETT (1946) differentiates between tertiary and non-infectious secondary palmar and plantar lesions, the present writer has been quite unable to do so with the cases met with in Sierra Leone, and in this paper all non-infectious lesions of the palms and soles are classed as late secondary. This has been done on account of the frequency with which infectious yaws lesions have been found to reappear in such cases if watched long enough, the common association with hyperkeratosis of the soft skin, *e.g.*, the ankles, and the non-development of a depigmented atrophic plantar skin in response to treatment as described by HACKETT.

Very little need be said about the manifestations of the disease, which are similar to those which have been repeatedly described elsewhere. But it seems clear that late secondary non-infectious lesions of the soles are much more frequent than in most parts of the world. In Sierra Leone these lesions account for the bulk of the non-infectious yaws cases, next in numerical importance being hyperkeratotic patches on ankles and wrists, but when other non-infectious secondary lesions exist plantar yaws is generally found also. Non-infectious, as well as infectious plantar yaws also, often coexists with secondary framboesiomata and condylomata, but uncommonly with tertiary lesions such as ulcers, gangosa, gummata and late bone and joint affections, accompanied by deformity. Serious tertiary lesions are not very common, but no doubt would have been more frequent if treatment had not been available at hospitals and dispensaries in Sierra Leone for many years past. Patients will go very many miles to the nearest centre for treatment of crippling tertiary bone and joint lesions and of ulcers, when they would not do so for secondary yaws. But in addition it seems probable that the development of tertiary lesions is inhibited by the frequency and persistence of late secondary foot yaws. Other lesions not often described but which are commonly associated with yaws in Sierra Leone are fascial contracture in the hands in adults leading to a condition closely resembling Dupuytren's contracture, and laxity of the ligaments of the knee joint causing hyperextension of the joint in the upright position usually in children. More or less painless effusions into the knee joint are not rare.

The relative frequency of non-infectious plantar yaws, as compared with other active yaws lesions, varies from place to place. In some chiefdom these foot lesions have accounted for 80 to 90 per cent. of the total yaws cases and in none have they fallen below 40 per cent. Most commonly plantar yaws is the chief lesion present in some 70 per cent. of the total cases. It is aggravated by trauma such as that entailed by working in swamps, and is therefore of considerable economic importance since those at all severely affected are debarrd

TABLE III

INCIDENCE OF APOX TYPES OF PRIMARY AND SECONDARY Y. LESIONS EXPRESSED AS PERCENTAGE OF PEOPLE TESTED IN EACH AGE GROUP OF THE POPULATION—ON A CHIEFDOM

Age group	0-4	5-9	10-19	20-29	30-39	40-49	50-59	60	Incidence all ages
Primary yew	1.14	2.8	0.34	1.9	6	0	0.0	0.0	
Secondary framboesomata	4.6	3.6	1.3	31	8.41	0.0	0	0.0	1.6
Granuloma on plantar	8.0	3	3.03	0.46	1.3		0.0	0.0	1.7
I see less—all types	8.64	1.40	4.1	1.20	4	1	0	0.0	3.7
Non-infectious plantar	8		3	11.3	11.3	7	3.9	1.4	1
Yew lesions—all types		13	13.3	1.3	11.9		3.9	1.4	1.7

This Table reveals two features of interest. Firstly primary and secondary framboesomata occur chiefly in the age period 0 to 9, granulomatous plantar yaws however is about equally common in the age groups 5 to 9 and 10 to 19 while the highest incidence of non-infectious yaws occurs in the later age group 20 to 39. This suggests a natural evolution of yaws lesions: first the primary infection soon followed by secondary eruption during the first decade; this is succeeded by infectious plantar yaws which persists into the second decade though in this decade framboesomata tend to die out; next follows non-infectious plantar yaws which reaches its highest incidence in the third and fourth decades; after the fourth decade yaws lesions tend gradually to disappear. In other words, yaws is a disease of slow evolution in this country and broadly speaking runs through its various overlapping primary and secondary stages over a period of 40 years or so.

Secondly, infectious yaws is relatively uncommon after the age of 30. The data from which Table III was compiled showed that 96.6 per cent. of the infectious lesions occurred in people aged under 30, and 89.4 per cent. in people under 40 years. In another large area comprising three chiefdoms with combined population of about 16,000 in this case examined in the dry season, the cases seen with infectious lesions fell into the age groups shown in Table IV. This Table gives on the percentage but the number of cases which fall into each estimated age group.

R D HARDING

from such occupations as climbing palm trees for the fruit, or swamp rice cultivation, and the worst affected are even incapable of clearing bush for ordinary upland farming. The problem is accentuated by the fact that plantar yaws has its highest incidence in young adults, particularly males. Thus in one community of 4,000 people, the incidence of this type of yaws was found to be 11.1 per cent in the estimated age group 20 to 39 (both sexes combined) against only 4.5 per cent in the remainder of the population. Sometimes the contrast is more marked even than this and one may find up to one-third of the young adult males with more or less disabling plantar lesions.

The age incidence at which infection occurs, and the sites of the primary lesions, are in accordance with the findings of most other observers. Thus in a questionnaire of some 400 people with a yaws history it was found that 35.5 per cent had been infected by the age of about 5, and 89.1 per cent by the age of 15, while only 10.9 per cent had become infected above this age. Most of those in the last category were women who had caught the disease from their children. In the same group of patients 87 per cent stated that the primary yaw appeared on the leg at or below the level of the knee, and this was confirmed by finding the old primary scar in the great majority of cases. No certain cases of reinfection have been met with.

The distribution by age groups of the more important types of yaws lesions as met with in mass diagnosis, has an important practical bearing on control. Table III shows the age incidence of four types of lesion in one community of 4,500 people examined during the wet season.

TABLE IV
INFECTIOUS YAWS CASES CLASSIFIED BY TYPE OF LESION AND AGE

Age group	0-4	5-9	(0-9)	10-19	20-29	30-39	40-49	50+	Total
Primary yaws	9	15	(24)	12	6	3	1	1	47
Secondary from boesiomata	40	42	(82)	20	7	5	1	4	128
Granulomatous plantar	13	22	(35)	46	17	4	5	5	112
Total	62	79	(141)	87	30	12	7	10	287

Note.—Probably some secondary stage cases exhibiting only a single framboesoma have been wrongly classified in this table as primary. The number of primary cases shown may therefore be disproportionately high.

In this community 89.9 per cent of the cases with infectious yaws were found in people of under 30 years. Thus it is evident that persons aged over 30 are relatively unlikely either to contract yaws or to suffer infectious relapses which might make them a source of infection to others. This is an important factor in a preventive campaign since it indicates that attention should be concentrated on the younger sections of the community and older people can be largely neglected.

There is no evidence that insects play any part in transmission. The genus *Hippelates* does not exist in Sierra Leone; house flies are not numerous and it is only occasionally that one sees them feeding on yaws papule. Biting flies also are not numerous except in certain localities where tabanids or tsetse are common. Yaws is in fact in Sierra Leone a house disease and new cases commonly become infected from other children in the same family or keeping in the same hut and all the evidence points to direct contact as the mode of spread.

ORGANIZATION OF THE CAMPAIGN

The yaws campaign arose out of the pre-existing sleeping sickness campaign and has been grafted on to the same organization. In the early stages of the former there were too many cases of sleeping sickness to be dealt with to allow of any systematic attack on yaws. By the end of 1942, however, the worst epidemic areas of sleeping sickness had been brought under control and it was possible to plan a campaign in which control of yaws became, in most areas, the main objective. It is appropriate to outline here the problem which presented itself. The main sleeping sickness area is contained in the west of Kailahun and Kono districts, so that it was necessary in any case to cover a large part of these districts to maintain sleeping sickness control. Sampling surveys of the whole of Kailahun and Kono, together with most of Kenema district had shown where sleeping sickness existed and where the incidence of yaws was at all considerable, or both, and it was decided to tackle the area so delimited. Though yaws was very prevalent in many other parts of Sierra Leone it was thought desirable to devote 3 or 4 years to the area mentioned in the first place, and to attempt to work out standardized methods of control which might later be applied elsewhere. The problem, therefore was to control yaws in a population of some 276 000 in whom the incidence of active yaws *i.e.* infectious yaws of all types plus incapacitating non-infectious yaws varied widely from place to place but averaged some 10 to 15 per cent. The paucity of road communications was an influential factor in determining the mode of attack, since it meant that travelling had generally to be done on foot and all equipment carried by porters and precluded the possibility of a medical officer supervising work running concurrently in several places. Salient features of the disease itself which had to be taken into practical account were (1) the lack of any practicable criterion of infection in dealing with large numbers except the observation of clinical signs of yaws (2) the long quiescent periods which occur spontaneously in the course of the disease or as the result of inadequate previous treatment when signs may be completely absent, but which may be followed by fresh eruption and (3) the tendency to spontaneous healing which occurs in the dry season. These features made it inevitable that mass treatment alone of cases presenting yaws lesions, even if repeated annually or oftener on two or three occasions would fail to effect anything like permanent control. The measures which have been attempted therefore comprise firstly mass diagnosis and treatment secondly the provision of permanent treatment centres strategically distributed and sufficiently numerous so that none of the

R D HARDING

population is further distant than a dozen miles from one centre or another, and thirdly, an organization of itinerant yaws attendants who tour the villages, spot yaws cases in their homes, and send them to the nearest centre for treatment. It has not yet been possible to implement these last two measures throughout the entire area.

Mass Diagnosis and Treatment

The political unit in the area is the chiefdom, with a paramount chief at its head, and this unit is found the most suitable on which to base diagnosis and treatment. Chiefdoms vary considerably in size but the average population is about 7,000.

Census—The first step is to list accurately the population of the chiefdom to be dealt with. This is done by a census team of about five African scribes in charge of a European administrative officer, who also builds the shelters in which diagnosis and treatment will take place and carries out propaganda. It is usually found necessary for him to carry out surprise visits by night to a number of villages already listed to check that no evasion is occurring.

Diagnosis—The medical team follows the census team into the chiefdom as soon as is practicable to commence diagnosis. It consists of a medical officer with about eight African sleeping sickness and yaws attendants. He calls in the villages of the chiefdom in rotation, examining 500 to 600 people daily. Diagnosis is made purely on clinical grounds. Children are stripped and examined all over, but in adults examination has to be confined to the exposed parts, that is, above the waist and below the middle of the thigh. Most attention is paid to seeking any signs of framboesiomata, condylomata, yaws macules and papules or ringworm-like lesions on the body generally, and of lesions on the soles and palms. The people are also questioned to elicit a history of any yaws eruption or incomplete treatment during the previous three years, but in many cases such a history is probably not disclosed. All persons with infectious yaws are taken for treatment together with those showing the dry skin lesions just described which are considered to be an indication of active yaws which may at some time become infectious. Those under about 30 years of age with non-infectious plantar lesions are also registered for treatment, as also are subjects above this age if the lesions are at all incapacitating, but otherwise treatment of non-infectious yaws in patients of middle age or over is left to the option of the patient.

Treatment—This is carried out at the same centres. Since injections are spaced at 5-day intervals to fit in with the treatment of any sleeping sickness cases receiving injections at the same time, it is usually convenient to arrange for treatment to proceed concurrently at two centres, and for this reason diagnosis is completed at two centres before treatment commences at either.

The drugs and dosages employed are described in a later section, and here it is sufficient to say that treatment has been mainly with a combination of acetylarsan and bismuth sodium potassium tartrate (B S P T) in watery solution administered both on the same day. Four double injections normally constitute the course. To give a typical instance a chiefdom of some 7,000 people is diagnosed and treated at two centres perhaps 10 miles apart, and some 500 cases may have been registered at one centre and some 350 at the other. Two days are spent in injecting the 500 cases at the first centre, on the third day the team treks to the second centre, on the fourth treats the 350 cases there, on the fifth treks back to the first centre, and on the sixth starts the second round of injections at that centre, and so on.

The method of administering the injections may be briefly described. The patients file up to the medical officer in the main shelter, who inspects their mouths for signs of stomatitis, enquires for any symptoms of intolerance or unrelated illness which might make a reduction of the normal dose desirable, and then enters the date and dosage to

be given on that particular treatment day in the register and on the patient card. The patient then passes on to receive his injections of acetylsalicylic acid and B.S.P.T. one into each buttock. All doses are graded according to body weight. Needles are boiled between every injection but syringes are only sterilized initially and not again unless blood should enter from vein. It is important to keep drug solutions covered, and to this end the solutions are placed in wide-mouth bottles fitted with tin lid perforated to allow the passage of a piece of fine rubber tubing. Into the free end of the tube is tied the hub of a hypodermic needle and the syringes are filled by fitting the needles to this.

Allowing for rest day and the time occupied in travelling, medical teams can diagnose and treat such children containing some 7000 people in 3 to 6 weeks, and then be read to pass on to another children. Since the census team can cover the ground more quickly than medical team it is most economical to have two of the latter working in conjunction with one of the former and with this combination it is found that the population can be dealt with at the rate of about 120,000 per annum in the more or less heavily populated regions containing 50 to 100 people to the square mile. Where the population density falls to 20 or 30 per square mile however the rate of progress declines nearly proportionately since it is distance rather than numbers which sets limit to the size of the unit team can deal with at any one time. With the active assistance of the chiefs, it is usually found that some 80 per cent. of the listed population can be obtained for examination, and that of the cases registered for treatment 85 per cent. or more receive minimum of six injections (i.e. three of each drug).

Drugs and Dosage Used

Neosphenamine, mapharside, sulpharsphenamine, sobits and bisoxyl have all been tried but none of these has been entirely satisfactory for one reason or another. Neosphenamine and mapharside, on account of expense and because the necessity for using the intravenous route restricts the number of injections that can be given in a day's work, particularly as a high proportion of the cases are small children. Attempts to reduce the extreme pain when mapharside is given intramuscularly by dissolving the drug in solutions containing glucose or novocaine, were not successful. Sulpharsphenamine, because toxic reactions have been said to occur with this drug in this country. Sobits, because its results have not seemed to be uniform and crops of stomatitis have occurred. Bisoxyl, because it settles quickly in suspension and consequently in the hands of African attendants the amount of drug in a given volume of the suspension injected is liable to variation. Acetylsalicylic acid and B.S.P.T. have therefore been in routine use until recently. Acetylsalicylic acid was adopted because of its rapid action, ease of administration, and alleged non-toxicity. It has also been strongly recommended by Wilson (1937). In practice in Sierra Leone, however its exhibition has not always been free from toxic sequelae, as described below. B.S.P.T. in watery solution was adopted for combination with acetylsalicylic acid because it had been in general use in Sierra Leone for many years, and on account of its cheapness, ease of preparation in the field, and lack of toxicity. No very extensive studies of its curative value appear to have been recorded in the literature but it was expected that its effects would be similar to those of bismuth salicylate which has been reported on so favourably by the Jamaica Yaws Commission (1933-1936). However a subsequent follow up of cases treated with B.S.P.T. has shown that results with this drug alone are very

poor, and bismuth salicylate in oil has now been substituted. The reason for the ineffectiveness of the B S P T used has since become clear. At the time of its adoption its bismuth content was believed to be high, but subsequent enquiry has disclosed the fact that the drug supplied to Sierra Leone contained only 4.34 per cent of bismuth metal, in conformity with Dr ALEXANDER's formula. In contrast, bismuth salicylate, B P, contains about 58 per cent bismuth. A combination of an arsenical and a bismuth salt has been used in preference to one drug alone on account of the great saving in time effected and the reduction in the number of attendances required from the patients.

The dosage tried out at the commencement of the campaign was based on that in use at the time at a local hospital, apparently with no ill effects. At this hospital a combined dose of up to 5 c.c. of acetylarsan (i.e., gramme 1.18 diethylamine acetarsol) and grain 5 (gramme 0.33) of B S P T was given up to twice weekly. Accordingly, a rather less intensive course of 5 c.c. acetylarsan and grain 4 B S P T (gramme 0.29) repeated at 5-day intervals was chosen for trial in the field campaign. The course comprised three such double injections, usually followed by a fourth injection of B S P T only. The full dosage was given to patients of over 100 lb. body weight (with the exception of the old, weakly, or otherwise sick) and children received a smaller dose proportional to their weight. For the first few months several thousands of patients were treated without any report of serious toxic reactions, then they began to occur in some numbers, and in one chiefdom there were 12 deaths probably attributable to the course among 1,350 cases treated. Symptoms comprised pyrexia, headache, nausea, vomiting, abdominal pain, diarrhoea, and prostration coming on usually within a few hours of the injection. Jaundice was a later sequela in an occasional case. Most of the reactions occurred after the third injection, indicating cumulative action. After this occurrence the dosage was reduced to a maximum of 4 c.c. acetylarsan (gramme 0.94 D.A.) and grain 3 (gramme 0.2) B S P T given at the same intervals, for a time all went well, but then toxic reactions with an occasional death again occurred, and the dosage was in consequence further reduced to 3 c.c. acetylarsan (gramme 0.71 D.A.) and grain 3 (gramme 0.2) B S P T.

During this time the low bismuth content of the B S P T used had not been realized, and though the type of reaction encountered pointed rather to the acetylarsan than the bismuth component as responsible, the possibility that the latter drug played a contributory part could not be excluded. To clear up this point some 400 patients were collected in the vicinity of the campaign's headquarters where they could readily be followed up, and were divided under three treatment categories:

- (1) acetylarsan alone, six doses of 4 c.c. (gramme 0.94 D.A.) each
- (2) B S P T alone, six doses of grain 4 (gramme 0.27)
- (3) a combination of acetylarsan and B S P T in 3 c.c. and 3-grain doses respectively, three double injections followed by a fourth of B S P T only.

All doses were given at weekly intervals. At each attendance patients were carefully questioned and their complaints recorded, their frequency is shown in Table V.

These results clearly incriminated acetylarsan and not B S P T, and showed that repeated doses of 3 c.c. to 4 c.c. were dangerously toxic in this part of Sierra Leone. Though some of the complaints of diarrhoea and vomiting might have been due to unassociated malaria, enteritis, and the like, the single death certainly, and the four cases of prostration very probably, were due to acetylarsan. The patient who died was a boy of about 13 years, whose body weight was 78 lb. and whose dosage was therefore 3 c.c. Two or 3 days after his second dose he "fainted" while on the family farm, but when next seen appeared quite well, however, his third dose was reduced to 2 c.c. as a precaution. On the occasion of the fourth injection he had no complaints, and

received 3 c.c. after which he remained apparently quite well until a week later when he collapsed suddenly on rising and died about 2 hours afterwards. On questioning the relatives, it then transpired that the "sunt" reported after the second injection had been much more than the term implied and that in fact the boy had been prostrated for 4 days after it. A postmortem was not possible.

Since acetylarsan is generally considered a relatively non-toxic drug, it may be that natives of Sierra Leone are peculiarly susceptible though whether on account of nutrition or some other factor it is not possible to say. However it is relevant to mention that in the treatment of sleeping sickness the one time

TABLE 1
REACTIONS OCCURRING DURING TREATMENT

Course	Number treated	Diarrhoea	Vomiting	Rheumatism	Prostration	Per cent of cases treated with reaction	Death
Acetylarsan 4 c.c. 6	109	14	3			11	1
B.S.P.T. 4 grains 6	no	0		1		13	
A 3 c.c. 3 B 4 grains 4	14	0	1		2	6	

Note.—4 c.c. acetylarsan contains grammes 0.93 diethylarsine acetylarsol, and 3 c.c. contains grammes 0.1. Grain 4 B.S.P.T. = grammes 0.17.

Nigerian standard course of three doses of amtrypol followed by five of try paramide, which in Nigeria is well tolerated, produced in Sierra Leone frequent and severe reactions with death-rat of about 5 per cent. as reported by LOCHRIS (1943) and HARDING (1945).

After this experience the maximum repeated adult dose of acetylarsan given in the mass campaign was limited to 2.5 c.c. and the standard course became four injections each of acetylarsan 2.5 c.c. (grammes 0.59 D.A.) and of bismuth salicylat grammes 0.25 given concurrently. Persons weighing 125 lb. or over received the full dose, and others smaller doses proportional to their body weight. In 63 patients treated by the writer with this course and closely observed and questioned by him no toxic reactions at all attributable to acetylarsan were noted, and in many hundreds of patients treated in dispensaries nothg more than an occasional mild case of stomatitis was reported.

R D HARDING

while immediate clinical results were excellent. It therefore appeared that 2.5 c.c. doses of acetylarsan were quite safe in this country, and that in the combination of this drug with bismuth salicylate in the dosages stated one had at last found a satisfactory course of treatment.

RESULTS OF MASS TREATMENT

The results to be described are those which followed a combined course of acetylarsan 4 to 5 c.c. (gramme 0.94 to 1.18 diethylamine acetarsol) \times 3, plus B S P T grain 3 to 4 (gramme 0.2 to 0.27) \times 4, used during the earlier period of the campaign. It is hoped to publish later the results of treatment with the acetylarsan-bismuth salicylate combination in a selected group of cases. These are somewhat better as regards response to treatment, but the effect on the trend of yaws in the community will probably not be very different owing to all the epidemiological factors involved.

APTED *et al* (1948) have already published a report on the results of a clinical and serological follow-up of small groups of cases treated in the course of this campaign, so that it is unnecessary to give here more than a brief summary. Table VI shows the clinical findings in some groups of secondary stage cases from different areas treated from 6 to 12 months previously with the acetylarsan—B S P T combination.

TABLE VI
RESULTS OF CLINICAL FOLLOW-UP OF YAWS PATIENTS TREATED IN SECONDARY STAGE

Original lesion	Area	Cases	Period since treatment	Season of re-examination	Per cent with lesions as under		
					Active yaws	Inactive plantar lesions	No signs
Infectious	I	75	1 year	LW ED MD	4.0	32.0	64.0
	II	104	1 "		2.9	—	—
	III	73	6-18 months		3.0	—	—
Non-infectious	I	108	1 year	LW ED MD LD	14.7	50.9	34.2
	II	253	1 "		10.7	—	—
	III	73	6-18 months		7.0	—	—
	IV	114	6		1.8	50.0	49.0

Notes.—Where blank spaces are left it was not recorded whether the patients had merely inactive plantar lesions or were completely clear. The term active yaws denotes all cases with framboesiomata or with marked plantar lesions accompanied by pain. Bone deformities and tertiary lesions, which were few, are not recorded.

LW = late wet season, ED = early dry, MD = mid-dry, LD = late dry

Owing to the influence of season on relapses (which this table exemplifies), the long quiescent periods which may follow treatment but be subsequently followed by a relapse, and the occurrence of spontaneous healing even in the absence of treatment, a clinical follow up conducted on one occasion only can furnish very little indication of the true cure rate in this respect serological findings are of more value.

The following Kahn results were obtained in 250 cases tested from 8 months to 2½ years after treatment with the acetylsalmon-B.S.P.T. combination in which the individual doses of acetylsalmon varied between 3 and 8 c.c.

Kahn one + to four +	+ weak or ±	Negative
38 per cent.	32.8 per cent.	29 per cent.

Results of Mass Treatment in Reducing Yaws in the Community

These results have been very different according to whether periodic mass treatment alone has been relied on or whether subsidiary measures have been employed in addition. They are therefore shown separately according to the system employed.

(a) *Periodic Mass Treatment Alone*—Table VII illustrates the results which have been achieved. It records the incidence of infectious yaws found in successive years. Only this type of yaws is shown because it gives a better index of reduction of infectivity in the community than if all types of yaws were included, and also because in the case of non-infectious types medical officers have probably differed in the selection of cases they have considered it desirable to record for treatment. With infectious yaws, however, difficulties of diagnosis hardly arise and all cases found are recorded.

TABLE VII.

PERCENTAGE INCIDENCE OF INFECTIOUS YAW. TIMES OF MASS TREATMENT AND TREATMENTS REPEATED IN SUCCESSIVE YEARS.

Area.	1941.	1942.	1943.	1944.	1945.
Gbese Jando	8.4 D	4.8 W	—	3.2 W	—
Mafindo	13.7 W	8.8 W	—	7.8 W	—
Kashoben	9.9 D	0.9 W	0.2 D	—	—
Mafindo	3.4 D	2.4 W	—	2.8 W	—
Birta	—	—	1.0 D	0.0 W	—
Kamara	—	—	3.8 W	3.1 W	5 W

Note.—W, diagnosed in wet season, D in dry season. The population of these areas is under about 5,000 each.

(b) *Periodic Mass Treatment Combined with the Provision of Permanent Treatment Facilities*—In the areas listed in Table VIII dispensaries were built about the time of the first diagnosis in 1942. The figures relate to areas whose boundaries lie within radius of 8 or 10 miles from the dispensary in each case.

R D HARDING

TABLE VIII

PERCENTAGE INCIDENCE OF INFECTIOUS YAWS AFTER ORIGINAL MASS TREATMENT IN 1942
ACCOMPANIED BY THE PROVISION OF DISPENSARIES

Area	1942	1943	1944	1945	1946
Mando	0 5 D	—	0 5 W	—	—
Kissi Teng	4 9 D	—	2 0 D	1 7 W	0 8 W
Kissi Kama	5 8 D	—	3 0 D	1 8 W	0 9 W

(c) *Mass Treatment Followed by Dispensary Facilities Combined with Home Visits* — In other chiefdoms mass diagnosis and treatment has been followed immediately by the provision of a yaws dispensary in each chiefdom, and in addition the attendant in charge of the dispensary has spent portions of his time on tour visiting the people in their homes and sending yaws cases discovered in to the dispensary for treatment. In the following chiefdom of about 7,000 people practically all the villages were visited in rotation. The 1944 figure relates to the incidence found at original mass diagnosis and treatment, the April, 1945, figure to the incidence found by the attendant on his home visits after the dispensary had been in operation for some months, and the September figure to the incidence found by a medical officer in a sampling survey of some 1,500 people inhabiting villages scattered throughout the chiefdom.

Chiefdom	Percentage incidence infectious yaws		
	Mid-1944 (wet season)	April, 1945 (early wet)	September, 1945 (late wet)
Dodo	3 4	0 6	0 0

In January, 1946, the yaws dispensary was closed and all work in the chiefdom ceased — due partly to shortage of staff and partly to the desire to discover what the effect of cessation of all treatment would be after 18 months' intensive work had reduced infectious yaws to zero. The following August a representative sampling survey of 1,670 people showed that the incidence had risen in 7 months to 1 7 per cent, so providing an excellent demonstration of the need for continued control of indefinite duration.

In this chiefdom the incidence of non-infectious yaws is also worth recording, since the examinations in 1944, in September, 1945, and in 1946 were all carried out by the same medical officer (Dr HUTCHINSON) at approximately the same time of year, and the figures are therefore comparable. They were 1944, 16 3 per cent, 1945, 6 3 per cent, 1946, 7 9 per cent. Non-infectious yaws, mainly plantar, was thus found to have shown a considerable reduction in response to continuously available treatment, though proportionately less than was the case with infectious yaws, and after cessation of treatment the succeeding rise was also less marked. In short, non-infectious yaws was slower to disappear and slower to reappear.

From the above three sets of figures it is quite clear that periodic mass treatment by itself has failed to produce a permanent reduction in infectious yaws. Since it is evident from Table VI, and from the results of the follow-up

conducted by APTED *et al.* (1948) that the incidence of infectious relapses in treated individuals during the next year or two following treatment is low the continuance of infectious yaws must be due to new infections and to recrudescences in cases which were latent at the time of previous diagnosis. Undoubtedly the latter factor is by far the more important of the two. Where in addition to mass treatment permanent facilities have been provided for the treatment of relapses, recrudescences, and new infections as they arise, the results have been very much better but since in fact few patients come voluntarily for treatment, either from laziness or the pressure of farm work immediately lesions appear there are always some cases to be found in the community and these continue to spread the infection. Undoubtedly the greatest reduction is obtained by the third method in which dispensary facilities are combined with home visits.

DISCUSSION.

The authors of yaws campaigns do not appear always to have been clear as to what exactly they intended to achieve, and it seems worth considering here what aims are both desirable and practicable in a primitive African society. Theoretically the possible objects of a campaign might be classified under four heads. (a) Total eradication of yaws from the community (b) elimination only of infectious types of the disease neglecting non-infectious types, with the aim of stopping transmission and so of preventing any further new infections (c) reduction of yaws to a point at which it does not cause any serious degree of suffering or loss of efficiency in the community or produce much invalidism, but continues to persist at a low endemic level in which most of the manifestations are mild and non-infectious (d) alleviation of symptoms by treating cases who seek treatment voluntarily and who may therefore only attend for one or two injections until lesions have healed, without attempting any radical reduction of the general mass of yaws in the community. Since the object in view will determine the methods of attack employed, one will also have to consider whether to limit activities to chemotherapy or to combine this with general hygienic measures designed to prevent infection. Finally one must decide whether to make an initial all-out effort to eradicate infection once and for all, or whether to plan a long term campaign of indefinite duration. These aims will now be discussed.

(a) *Eradication of Yaws*—Under this heading it will be convenient first to dispose of the two questions just raised. As regards the supplementation of chemotherapy by general hygienic measures, it may be said at once that if they were to have any marked effect such measures would have to be employed on too widespread and costly a scale to be feasible as part of a yaws campaign *per se*. Probably the conditions most conducive to the spread of yaws are close crowding of naked children in hot, humid, and dirty mud huts, together with the absence of personal cleanliness, and it is in just such conditions that the bulk of the

R D HARDING

population of West Africa lives. Their correction involves the wide fields of public health, education, economics, and native tradition, and it will be many decades before the general standard of living can be raised sufficiently to influence appreciably the incidence of yaws. Chemotherapy must therefore remain for the present the chief weapon in a yaws campaign in West Africa.

In regard to the possibility of eradicating infection by a short-term intensive treatment campaign, some of the figures already given supply a fairly adequate commentary. Some relapses after treatment will always occur, there will be recrudescences, often at long intervals, among people who, though already infected at the time of examination, then showed no signs of yaws, it will never be possible to inspect quite 100 per cent of a population some members of which are always on the move, finally, in Africa it is impossible to isolate a community from outside influences, and once a large proportion of sources of infection, reintroduction of the disease from outside, or from inside by even an occasional infectious relapse or recrudescence, may lead to a serious epidemic. The course of events in Western Samoa affords a good illustration of such an occurrence. LAMBERT (1936) records that as a result of an intensive campaign in that country during the years 1923 to 1926, in which 25,000 persons out of a population of 40,000 received treatment, the incidence of primary and secondary yaws was by 1926 reduced almost to zero and it seemed that infectious yaws was on the point of disappearing. However, owing to political disturbances, the campaign then ceased, with the result that by 1930 and 1931 primary and secondary yaws began, in LAMBERT's words, to sprout like mushrooms in every direction.

Even if the eradication of yaws were possible its achievement would, in the writer's opinion, be not without danger owing to the at least partial immunity it appears to confer against syphilis, so that the disappearance of yaws might be followed by a big increase in the incidence of syphilis. This point of view has been argued by BLACKLOCK (1932), and is supported on the experimental side by the cross-immunity induced between yaws and syphilis by TURNER *et al* (1947) in rabbits, as by similar observations by other workers using monkeys. VAN NITSEN (1944), on the other hand, basing his opinion on his extensive experience in the Belgian Congo, does not consider the risk very great—certainly not serious enough to contraindicate an attempt to eradicate yaws. Yet the present writer's experience of the prevalence of syphilis in parts of Nigeria where yaws does not exist, its comparative rarity in some other parts of Nigeria and in Sierra Leone where yaws is endemic (though gonorrhoea is ubiquitous), and the absence of a yaws history in such cases of syphilis as he has come across in the rural areas of the latter colony, conveys to him at least a cogent warning. HACKETT's (1947) comparative figures obtained in Uganda indicating the prevalence of syphilis at Masaka where yaws is uncommon, and its infrequency at Lira where yaws is rife, point the same way. Since yaws

is a disease usually contracted in childhood and syphilis one contracted usually in adult life, the presence or absence of yaws is likely to determine the incidence of syphilis rather than vice versa.

(b) *Elimination of Infectious Yaws Only*.—At the commencement of the campaign it was thought that to confine treatment to those cases which, when seen, were suffering from an infectious stage of the disease would, by cutting off the source of fresh infections, be as efficient in reducing the subsequent incidence of yaws in the community as would the treatment of all forms of yaws. Observation of untreated non-infectious secondary yaws cases, however showed this assumption to be false, owing to the liability of such persons (at least those aged under 30) sooner or later to develop infectious eruptions, and so provide fresh sources of infection. Thus it is common for pseudo-granulomatous infectious lesions to develop on the soles in cases which have shown, perhaps for years, only non-infectious plantar yaws, and not uncommon for weeping papules to arise in long standing areas of hyperkeratosis on the ankles. Infectious lesions do not appear to recur in cases which have reached the tertiary stage, but such form a comparatively unimportant minority in Sierra Leone.

(c) *Reduction of Yaws to a Low Endemic Level*.—This has now become the true objective of this campaign and has been achieved in some areas by the methods already described. In such areas people hobbling along with crab yaws are now seldom to be seen, it is rare to see chronic bone and joint disease with deformity and the general health of the community has improved very markedly. New infections occur but are less common than formerly.

(d) *Hospital or Dispensary Treatment of Patients who Attend Voluntarily in the Absence of Mass Diagnosis*.—Undoubtedly such institutions have in the course of years had a considerable effect on the prevalence of infectious yaws but only in their immediate vicinity and the method is certainly wasteful of drug and in the long run expensive because most patients only attend for from one to three injections, in consequence of which they are liable to repeated relapses. Many active yaws patients have been met with who have received one or two injections yearly for several years, sometimes amounting in the aggregate to 15 injections or more, whereas if they had received a course of six or seven injections of a potent drug at the beginning it is probable that the majority of them would not again have suffered from active lesions.

Two other theoretically possible modes of attack should be briefly mentioned. Firstly that of carrying out serological tests on every person in the community and treating all found positive however even using the comparatively simple Id test which in any case would give very doubtfully reliable results in the hands of African assistants, the pace would be too slow for dealing with a large community. Secondly that of giving every person in the community suppressive treatment regardless of whether signs of yaws could be found or not. Apart from the probable reluctance of apparently healthy people to receive injections, one would be faced with the problem of

P. D. HAPDING

whether to give one or two injections only, or alternatively, a longer course aimed at being curative. There is ample evidence that one or two injections would be followed by numerous relapses among the infected section of the community whereas to give six or seven injections to every person would be altogether too big an undertaking.

CONCLUSION

In Sierra Leone the most practicable method of controlling yaws has been found to be in three stages. First mass diagnosis and treatment. Second immediately afterwards the provision of permanent treatment dispensary spaced at strategic intervals and sufficiently numerous to avoid the necessity for any inhibition of the area having to walk more than 10 miles for treatment. Third home visits by a yaws attendant to detect cases in their villages and send them to the dispensary for treatment.

The provision of numerous permanent dispensaries is undoubtedly the most important of the three stages, and has the advantage that other endemic diseases can be dealt with by them if desirable at the same time, but in Sierra Leone it is very doubtful whether they would prove a success unless preceded by mass treatment. Mass treatment both reduces the amount of yaws at one stroke to a manageable level and, equally important, accustoms the people to the necessity for an adequate therapeutic course. At the more successful dispensaries and with the help of the local chiefs, 80 or 90 per cent of patients attend for six injections. Had these dispensaries been opened without previous mass treatment it is unlikely that half the patients could have been persuaded to attend for more than two or three injections.

A combination of acetylsalicylic acid and bismuth sodium potassium tartrate (Dr ALEXANDER'S formula) was used in mass treatment during the earlier part of the campaign, but the latter drug was shown to be relatively inactive and bismuth salicylate was substituted. Acetylsalicylic acid in repeated doses of 3 c.c. or more was found to cause serious toxic reactions in a number of cases, and the course eventually adopted comprised four injections each of acetylsalicylic acid 2.5 c.c. (gramme 0.59 diethylamine acetate) and of bismuth salicylate gramme 0.25 given concurrently at 5-day intervals. Experience with B.S.P.T. emphasized how essential it is to know the bismuth content of the particular preparation employed if this drug is used, since its composition varies greatly according to method of manufacture.

Results of the campaign using the acetylsalicylic acid-B.S.P.T. combination are described both in groups of treated patients and in whole communities. The degrees of reduction of yaws in the community are compared after (a) mass treatment alone, (b) mass treatment followed by the provision of dispensaries, and (c) these two measures with the addition of home visiting and inspection.

The aims of a yaws campaign which may be considered both desirable and practicable in a primitive African society are discussed.

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SEVERE ANAEMIA IN INDIAN SEPOYS (REFRACTORY TROPICAL MACROCYTIC ANAEMIA)

BY

R. PASSMORE, D.M., Major, I.M.S.

The purpose of this paper is to give an account of a series of cases of severe anaemia, occurring amongst sepoys recruited from the Burma battle area. The majority of the anaemias were macrocytic, but they differed from the general description of tropical macrocytic anaemias in that for the most part there was no response to accepted nutritional remedies and in many cases there was no evidence of associated malnutrition. It does not appear possible that a simple lack of Castle's extrinsic factor or other dietary factor could alone be responsible for these haemopoietic failures. The paper is based on an analysis of 127 case sheets of patients whose haemoglobin level had at one time fallen below 4.0 grammes per 100 ml. All the patients were treated during 1943 and 1944, at two large Indian general hospitals in Calcutta and Lucknow, where they had been received as casualties from hospitals in the active service areas of Assam and Eastern Bengal.

Broadly speaking, anaemias in the Indian army fell into two groups. Firstly, there was a group in which healthy bone marrow was unable to produce a sufficiency of circulating haemoglobin, either because of lack of supplies of essential blood-forming components in the diet or because of excessive demands due to the destruction of blood from some extraneous disease. Such cases may be called symptomatic anaemias, since the bone marrow remains undamaged. The principal causes of these anaemias amongst sepoys were malaria, defective food intake and megalostomiasis, but many other factors were occasionally contributory. In general, these anaemias were extremely

common, but they were rarely severe and they responded satisfactorily to treatment. On account of their frequency these cases presented a most important military and administrative problem, but inasmuch as a causal factor could usually be found and recovery was generally satisfactory after its eradication, they did not present great clinical difficulties. Secondly there is a group in which the bone marrow itself is diseased and is unable to produce adequate amounts of blood, despite an apparent sufficiency of nutriment materials and the absence or the eradication of any disease factors, making for excessive blood destruction. These may be called the refractory anaemias, as there is evidence of a general bone marrow failure. For the most part they resulted in severe prolonged illnesses, resistant to treatment and with a considerable mortality. The majority were macrocytic. These refractory anaemias were not so common as to affect seriously the fighting strengths of units. They were, however, rough more numerous than in European practice. Further since each case remained several weeks, or often months, in hospital, they provided many clinical problems and were a constant source of difficulty to base hospital staffs.

It is realized that this distinction is not hard and fast. In particular if a symptomatic anaemia is allowed to proceed for a long period without treatment, bone marrow changes may develop and it may slip imperceptibly into the refractory type. Further these severe anaemias do not present a uniform pathological picture. The majority of the cases were received during rush periods and investigations had to be limited to essentials necessary for diagnosis and treatment. The problem of treatment fell mainly on Central Command, since the majority of base hospitals were within their area. A wide experience of the condition was gained by many hospitals and centres and an opportunity arose for an exchange of views at an anaemia conference at Agra organized by Colonel B. E. SCHLENGER, Consultant Physician to the Command, in November 1944. This was attended by over 40 officers mostly medical specialists and pathologists. The Proceedings of the conference, which were published and are obtainable from the D D M S Central Command, Agra, contain a valuable summary of facts and current ideas.

Incidence—The data about the incidence of severe anaemia (Hb. less than 4.0) amongst different types of troops are presented in Table I.

Similar data for the whole population of the Fourteenth Army are not available here and exact comparisons cannot be made. It is thought that these figures confirm a general impression that the incidence of these anaemias is evenly distributed amongst different classes of sepoys. All types are liable to suffer. The well built NCO of some years service belonging to the "martial" classes and the undersized, recently enlisted pioneer appear to have had a similar susceptibility.

No figures are available as to the number of cases of severe anaemia admitted to other hospitals or to the total number of troops at risk. The exact incidence of the condition cannot be given. A reasonable guess is that one out of between

1,000 and 5,000 sepoys in the Fourteenth Army developed severe anaemia. The condition was practically never seen in British troops. Thus LEISHMAN (1944), analysing the cases of 11,645 patients admitted in a single year to a British base general hospital, of whom 2,819 had malaria, stated that chronic anaemia was rarely seen even in patients who had had as many as 15 attacks of malaria in as many months.

TABLE I

	Percentage of cases
Infantry regiments	18
Ancillary troops (R.I.A.S.C., I.A.M.C., I.E., etc.)	60
Pioneers and followers	22
Total service	
Less than 1 year	5
1 to 2 years	36
2 3 "	33
3 " 4	13
Over 4	13
Service with the Fourteenth Army	
Less than $\frac{1}{2}$ year	14
$\frac{1}{2}$ to 1 year	36
1 " 2 years	34
Over 2	16
Hindus	72
Muslims	27
Christians	1
Home Province	
Punjab	38
United Provinces	16
Madras	9
Bombay, N.W.F.P. and Nepal	5 each
Bihar	3
Gwalior and Rajputana	2
Kashmir, Orissa, Bengal, Baroda	1

ASSOCIATED DISEASES

The great majority of cases of anaemia occurring in the Indian Army could be indirectly associated with an inadequate military hygiene. This was shown by the history of events following the raising of the general level of hygiene. With improvement in rations and well-developed air supplies, good anti-mosquito measures and discipline, suppressive mepacrine, supplementary feeding of recruits, higher standards of cleanliness in units' lines and the introduction of DDT, cases of anaemia in hospitals became fewer and fewer. Finally,

in 1946 amongst the army of occupation in South East Asia, anaemia ceased to be an important medical problem. Further there was a definite seasonal incidence, cases being much more numerous during and just after the monsoon, a time when the application of hygienic methods is always difficult. Attempts to enter into details and assess the importance of individual hygienic factors is much more difficult. Table II gives a list of associated diseases found in the 127 cases.

Malaria.—As the table shows, the majority of the men presented evidence of a recent malarial infection. Almost all had served in highly malarious areas. They had been supplied with mosquito nets and suitable clothing, long trousers and sleeves being worn at night, but only a few men at the end of the series had been on suppressive mepacrine. Whilst it is certain that most of the men had been infected, it was by no means clear that malaria was the cause of the

TABLE II
DISEASES ASSOCIATED WITH SEVERE ANAEMIA (127 CASES).

	Number of cases
Malaria: protocol diagnosis, 83; clinical diagnosis, 33	96
Malnutrition	84
Ancylostomiasis (excluding 17 insignificant infections)	10
Chronic amoebic dysentery	9
Other conditions (tuberculosis, 3; leukaemia, 1; kala-azar, 1)	6

anaemia or how it operated. The majority had had standard anti-malarial therapy in forward hospitals. It is certain that direct destruction of the red blood corpuscles by the parasites was only an occasional factor. In only a very small number of cases could parasites be found in large numbers. It was more usual to find a few after many failures. Some could be attributed to latent malaria. The following is an example.

Naik S. H., driver in the R.I.A.S.C. was evacuated from his unit on 14 October 1944 complaining of weakness and giddiness of 1 month duration. On examination he was afebrile, his nutrition was recorded as fair and his spleen was not palpable. Three examinations of the blood by thick films failed to detect malarial parasites. The Hb was 5.7 grammes per 100 ml. He received dietary and symptomatic treatment only and was sent back to base hospital. After being afebrile for over a month, he developed severe rigors and was acutely ill. Parasites (*Plasmodium vivax*) were found in the blood, and the Hb level had fallen to 2.6 grammes. He made an eventful recovery following anti-malarial therapy and transfusion.

Such patients in whom all overt signs of malaria, except anaemia, were absent for long periods, provided ample justification for a medical directive that all cases of anaemia coming from malarious areas should have anti-malarial treatment, whether parasites were found in the blood or not.

R PASSMORE

Although the importance of recent malaria must not be under-estimated it was by no means universally present. Four patients who gave no history of malarial fever and in whom malarial parasites were not found in life, died, and no evidence of past malarial infection could be found either in the spleen or bone marrow.

Malnutrition—Forty-three per cent of the cases had evidence of malnutrition. The chief manifestations were emaciation and nutritional diarrhoea, not associated with inflammation of the bowels. Nutritional glossitis and stomatitis and occasionally nutritional hyperkeratosis, were seen. The picture was that of malnutrition in general and has been described in a previous paper (PASSMORE, 1947). Patients with severe nutritional breakdown were common in both hospitals at the time, but only a minority of these developed a severe anaemia. Further, the majority, 57 per cent of the patients with severe anaemia, presented no evidence of malnutrition. Many died after weeks in hospital with no signs of deficiency diseases and with no obvious wasting of muscle or fat. Thus the epidemiological association of severe anaemia and malnutrition was close throughout the Burma campaign, but the clinical association was much less marked. Both conditions were frequently found in an advanced and even fatal state, quite independently of each other.

The official diet for these men in the forward areas is given in Table III

TABLE III
FIELD SERVICE SCALE (1943)

Atta or rice	20 oz	Onions	2 oz
Dhal	2½	Potatoes	4
Ghee	3½	Vegetables (fresh)	4
Milk, tinned	2½	Mutton	6 "
Sugar	2½		

Most of the anaemia cases occurred during the most difficult period of the war from the supply viewpoint. Many men had been in the siege of Imphal on the dry ration of cereal, dhal, tinned milk, sugar and tea had in almost all cases been regularly supplied. But many sepoy's stated that the vegetable ration often of very poor quality, was only received once or twice a week and the meat supply two or three times a month for long periods. Forty per cent of the patients were strict vegetarians. This is a slightly higher incidence than in the Army in general. However, for the period immediately before admission, the great majority of the cases were in obligatory vegetarians.

Ancylostomiasis—Hookworm ova were found in 26 per cent of the stools by routine examination of three specimens by a flotation method. In half of these cases the number was small and not associated with iron deficiency.

anaemia. In only one case were more than a dozen worms found in the bowel at postmortem. The low incidence of hook worm infestation in these cases, and in the Fourteenth Army in general can be attributed to efficient worming in base depots and the prolonged wearing of boots. It would also suggest that the period of 2 years, usually given as the life span of the worms, is possibly too long.

Chronic Amoebic Dysentery—This was present in nine of the cases. Except in its indirect effect on nutrition, it was probably not responsible for the anaemia. The widespread use of sulpha drugs greatly reduced the importance of bacillary dysentery and this disease was not considered to be a contributory factor in any case.

CLINICAL PICTURE

The clinical signs were not striking. Progressive weakness was often the only complaint. Those in whom there was an associated nutritional failure presented the picture described in a previous paper (PAINSON, 1947). But many of these men maintained a satisfactory nutrition throughout a long illness. It was surprising how well adjusted the patients became to the low haemoglobin level. It was usually impossible to keep those with Hb between 3 and 4 grammes in bed. Yet no case of sudden heart failure occurred. One man only had reported attacks of anginal pain, lying in bed, when his Hb level was 7.5 grammes. However he made an otherwise uneventful and complete recovery. Haemic murmurs were usually heard. In fatal cases, the end was usually gradual with progressive weakness and terminal heart failure with oedema. Subcutaneous haemorrhages and severe uncontrollable oral haemorrhages were common terminal events.

Fever was almost universal. It was characteristically quite irregular. Occasionally sustained temperature charts resembling those in enteric fever were seen. Numerous blood cultures were sterile and there can be no doubt that the fever occurs in the absence of infection.

Test meals gave quite irregular results. Hypochlorhydria was not a feature of the cases. In no single case was there any evidence of spinal cord lesions. Subacute combined degeneration of the cord is undoubtedly extremely rare in Indians.

Haematology—Full blood counts including haematocrit measurements, were made on 83 cases. In Calcutta these were carried out at the School of Tropical Medicine through the courtesy of Dr C. R. DAS GUPTA. Standardized methods and apparatus were used (NAPIER and DAS GUPTA, 1947). In Lucknow only the ordinary military hospital apparatus was available and this did not include an electric centrifuge. However it was found that a high-gearred hand centrifuge and an improvised machine made from a table fan, packed the red cells in anæmic blood efficiently. Although prolonged and fast centrifuging is essential for packing normal blood, worker in the tropics without a modern

R PASSMORE

centrifuge can obtain haematocrit values with an error of less than 5 per cent from anaemic bloods, provided the haemoglobin level is below 6 grammes. The figures are given in Table IV.

The classification is based on the data obtained by NAPIER and DAS GUPTA for normal Indians. These workers found that the mean corpuscular volume (M C V) varied between 80 and 100 cu μ and the mean corpuscular haemoglobin (M C H C) was over 30 per cent in healthy Indian subjects. Accordingly all bloods with M C V values under 80 are classified as microcytic, between 80 and 100 as normocytic and over 100 as macrocytic. Similarly, bloods with M C H C values less than 30 are considered hypochromic and over 30 orthochromic.

The first and obvious conclusion that can be drawn from a study of the data presented in Table IV is that the patients present a mixture of all common types of anaemia. They do not conform to a uniform haematological pattern. Several other points of interest arise. Thus it will be seen that 37 patients in

TABLE IV
TYPES OF ANAEMIA

Macrocytic	Orthochromic	31	51
	Hypochromic	20	
Normocytic	Orthochromic	16	28
	Hypochromic	12	
Microcytic	Orthochromic	—	5
	Hypochromic	5	

all were classified as hypochromic, with M C H C below 30 per cent. Only these presented any evidence of iron deficiency. In this group of 37, 16 were suffering from ancylostomiasis, leaving only 21, or less than 25 per cent of the total, with any evidence of a primary iron deficiency. Further, the standard of normality for the M C H C set by NAPIER and DAS GUPTA is high. If a lower level of normality is taken as 28, the number of hypochromic anaemia cases is reduced from 37 to 25 and of these 13 had evidence of ancylostomiasis, leaving only 12 or 14 per cent of the total cases in which a diagnosis of primary iron deficiency can be made. It is thought that this figure leaves no room for doubt that there is no serious deficiency of iron in the Indian sepoys' diet. Further, contrary to current opinion, the majority of cases of malarial anaemia, being orthochromic, present no evidence of iron deficiency. Presumably the iron liberated from the haemoglobin of the broken down corpuscles is not excreted, but is available for the resynthesis of the pigment.

It will be seen that the total in the macrocytic group forms 61 per cent of the whole. Thus more than half the cases can be described as tropical

the bone marrow will regain its normal function in time after due rest, provided that infections are eradicated and the patient can be kept alive. Many cases have been seen in which a slow resumption of normal haematopoietic activity occurred after weeks of failure to produce normal blood. Others have slowly deteriorated and developed complete aplasia. The most essential part of treatment is to assure that there is enough circulating haemoglobin to keep the vital functions of the tissues going. Only adequate blood transfusion can achieve this.

TREATMENT

The general and dietetic treatment of severe anaemia and malnutrition are very similar. A full description was given in a previous paper on deficiency diseases (PANDIT, 1947) and need not be repeated. The following account is confined to a description of specific haematological treatment, but it may be stressed that these can be of little value in the absence of an adequate diet and with uncontrolled infections.

Blood Transfusion—Experience has shown that all cases with haemoglobin level of 4.0 grammes or less require transfusion. Below this level, there are no contra indications to transfusion provided adequate facilities are available. However severely ill or no matter what secondary complications exist, transfusion is indicated. Fever in particular is an indication and the irregular temperature so often associated with severe anaemia when the Hb. level is between 2 and 3 grammes, often ceases promptly after transfusion. There is a natural fear that such dangerously ill patients may suffer from a severe febrile reaction, which will prove fatal. Such a risk exists and deaths have been seen following quickly upon transfusion. However the risk of not transfusing is greater and provided adequate facilities exist blood should be given in every case. With the greatly improved methods of transfusion, risks have become small. The essential factor in a blood transfusion is the replacement of circulating haemoglobin. No satisfactory evidence has been forthcoming that whole blood has any direct stimulating effect on bone marrow. If this point is realized, certain facts about dosage follow. Five hundred ml. of whole blood should contain above 70 grammes Hb. If this is given to a person with a blood volume of 6 l. the Hb. level will be raised 1.16 gramm per 100 ml. Such a rise can only result in a small clinical improvement. For severe anaemias the dosage of blood must be two to four times this amount, i.e. 1,000 to 2,000 ml. per transfusion, and this dose may need to be repeated after a few days. Results with smaller doses have been disappointing and uneconomical. In giving large blood transfusions to severely ill patients, the slow drip method developed by MURPHY and LUKWICK (1935) must be used. Every bottle of blood, 500 ml. should take from 4 to 6 hours. In large transfusions, if a continuous watch is taken to see that the circulation is not overloaded, there is

little risk. Febrile reactions and even rigors are no indication for stopping, provided the circulation is well maintained. With suitable sedatives and attention to nursing details, many patients can sleep through the greater part of a 24-hour transfusion. It is generally advisable to tie a cannula into a vein. The patient can then move about in bed and splints are rarely necessary.

Reactions to transfusions have become both less frequent and less serious. Early in 1943, nearly every patient suffered some febrile reaction and severe reactions were frequent. At the end of 1945 reactions were reduced to about 20 per cent and were seldom severe. This improvement was gradual and corresponded with the development of the Army Transfusion Services. The medical patient is particularly susceptible to pyrogens (much more so than the surgical) and the gradual improvement in the quality of rubber tubing, citrate, glassware and in improved methods of cleaning and assembling apparatus by reducing the pyrogens was probably responsible for the reduced number of reactions.

In all, these patients received 545 bottles of blood. The 48 fatal cases had 240 pints, an average of 5.3 pints per man, and the 79 who recovered had 305 pints, an average of 3.8 pints per man. It is probable that more lives could have been saved had more blood been available. But anyone familiar with the difficulties of conducting blood banks in India will realize that great credit is due to those responsible for supplying such quantities as were obtained. The difficulty was increased by the fact that the greatest demand for blood from the anaemia wards occurred in September and October, 1944, when we had 21 deaths. At this time heavy fighting was taking place on the Burma border and surgeons at the front were naturally given a priority of supply, blood collected in base areas being sent forward by air.

Many of the anaemic patients required repeated transfusion. One man had as many as 35 pints spread over a period of 4 months. At first he seemed quite unable to manufacture any blood for himself, but slowly his bone marrow recovered and finally he appeared able to maintain a normal level without transfusion.

Liver Therapy—A course of liver therapy was given to 56 patients all with evidence of macrocytosis. The dose given was 4 ml. daily for 6 days subcutaneously. The majority of patients received an Indian preparation known as 'I.C.T.' which had been tested against cases of pernicious anaemia in England and shown to be potent (CAMERON, 1944). A few patients received such well-recognized preparations as exatope, pernaemon and neohepatex. Of this series, 38 cases (68 per cent) showed no sign of improvement, either clinical or haematological, within 10 days of the completion of the course. Of the remaining 18 in whom improvement was observed, 10 were receiving concomitant treatment such as transfusions or anti-malarial therapy. In only eight (14 per cent) was there any improvement under liver alone, even in these the possibility of spontaneous recovery cannot be ruled out. In no case were improvements comparable to those observed in liver therapy of pernicious anaemia. Reticulocyte counts of 10 to 15 per cent were the maximum observed. There can be no doubt that the liver preparations available had no specific

effect on the severe macrocytic anaemia seen in the Fourteenth Army comparable to liver on pernicious anaemia cases in Europeans. Sometimes definite non-specific effects probably occurred.

Yeast Therapy—The great majority of the patients had yeast preparations, Marmite, vegamite and a concentrated dry yeast pill were used. In not one single case was the writer satisfied that they were of any value. This is possibly accounted for by the exceptionally good dietary of both hospitals. At all times unlimited fresh milk, curds, fresh vegetables, fresh and tinned fruits, chicken and mutton were available. In the writer's experience yeast preparations are of no practical value for supplementing a first-class natural diet in the treatment of either anaemia or malnutrition. Should, however a hospital be unable to supply adequate amounts of protective foods in the diet, then there can be no question that yeast is a most valuable partial substitute.

DISCUSSION

Most standard textbooks of medicine and haematology dismiss tropical macrocytic anaemia in one page. It is described as a disease caused by the lack of an essential dietary factor probably Castle's extrinsic factor and curable by liver or marmite. This simple story is based on WILLS' (1931 and 1933) experience of pregnancy anaemias in Bombay. The cases of macrocytic anaemia described in this paper do not fall within this concise description. They differ in that most of the patients proved resistant to dietary therapy and that many showed no clinical evidence of malnutrition. A wider conception of the condition, involving considerations in addition to that of a simple deficiency disease, is necessary.

The problem of the aetiology of these anaemias is of great interest. There can be no doubt that in the majority of cases the disease was immediately attributable to recent conditions of service and was usually precipitated either by a recent attack of malaria or by a period of subsistence on inadequate food. The disappearance of severe anaemia following the great improvements in Army hygiene is strong evidence to support this view. But that these precipitating factors were not the whole story is shown by the freedom of British troops from the disease. This freedom naturally led to a search for any distinction between the conditions of service of the British and the Indians. Both were exposed to the same infective hazards. The standard of hygiene, anti-malarial discipline and unit cleanliness was certainly no higher in British than in Indian units. All the sick received similar treatment at the same forward hospitals. At the worst Indian troops subsisted on a diet of atta, rice and dhal, and British troops on biscuits and bully beef. In both cases tea with milk and sugar being usually available. Many persons, including the Consultant Physician to the Force (MARRIOTT 1944), considered that the immunity of British troops to anaemia might be attributable to the higher amounts of animal protein consumed.

Although minor degrees of anaemia may be attributable to this cause, too great an importance should not be attached to the haematopoietic properties of bully beef. That a simple nutritional macrocytic anaemia exists, and that it was common amongst the troops in the Burma Army, there can be little doubt. An excellent description of such an anaemia as it occurred in sepoy serving in Iraq is given by TAYLOR and CHUTTANI (1945). But it is extremely doubtful if any dietary deficiency operating only for a few months can give rise to anaemias of the severity of the cases described in this paper. Modern literature is full of accounts of starvation and gross malnutrition. Severe anaemia does not feature in these. Both in Belsen (MOLLISON, 1946) and in the Bengal famine, anaemias of this degree were conspicuously absent. The cause for these haematological failures must be sought farther afield than in the dietary inadequacies of the previous months. As regards malaria, it is equally difficult to attribute the condition solely to recent attacks. Although improvements were subsequently effected both in the treatment and prevention of malaria, yet at the time anti-malarial precautions were enforced and adequate supplies of anti-malarial drugs were available and were used. The British troops, who suffered equally severely from acute malaria, did not develop similar anaemias. This fact would appear to rule out the particular type of malaria prevalent on the Burma border as the sole or even the principal cause of these conditions.

Both the clinical and epidemiological evidence would suggest that we must look back into the past, before the beginning of the war, for the ultimate origin of these anaemias. It is probable that certain sepoys entered the Burma campaign with a defence mechanism less well equipped than normal to withstand the haematological strains imposed by a period of defective rations and a simultaneous malarial infection. In the great majority of both British and Indian men at risk the bone marrow reacted vigorously and, as soon as the extra strain was removed, the blood was returned to normal. In this small minority the marrow appeared unable to react adequately and a long period ensued of impaired function often terminating in physiological failure sometimes with complete aplasia. This inadequacy of the bone marrow might be the result of repeated previous strains in childhood and youth, probably either from long standing nutritional defects or repeated malarial infections.

SUMMARY

- (1) An account is given of 127 cases of severe anaemia with haemoglobin levels below 4.0 grammes occurring amongst Indian sepoys evacuated from the Burma campaign.
- (2) The majority had had recent malarial infections, and just under half presented evidence of a defective nutrition. Ancylostomiasis was not frequent.
- (3) A variety of haematological types occurred, but the majority were macrocytic anaemias.

(4) Many of the patients differed from cases of tropical macrocytic anaemia as usually described in that they were refractory and did not respond to either yeast or parental liver therapy. Repeated blood transfusions were found to be the most important therapeutic agent.

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STANDARDIZATION OF DABOIA AND COBRA ANTIVENINES *

By

R K GOYAL, D Sc (PARIS), Ph D (EDIN), M R C P (LOND), F R S E,
From the Central Research Institute, Kasauli

Snake venoms differ markedly in their chemical composition and physiological properties. There are consequently a large number of specific antivenines, and it is not legitimate to titrate different venoms against one antiserum. The Health Organization of the League of Nations has not, therefore, attempted to set up an international standard for the titration of anti-snake venom sera. It should, however, be possible to have a uniform test for the titration of any one type of antivenine.

IPSEN (1938) mentioned that for the titration of European viper antivenines, four laboratories used three different species of experimental animals, the route of injection and the basis of estimation of potency also varied. Working on European viper antivenines, he indicated that the strength of the sera appeared to vary in different animals injected by the same route and in the same animal injected by different routes, but no particulars were given. In the absence of comparative tests in different animals it is, therefore, not advisable to select one particular species of animal at random for standardization purposes.

No detailed observations have been published regarding the titration of daboia and cobra antivenines in different animals by different routes and in the same animal by different routes. This work was, therefore, undertaken to determine (1) whether results similar to those of IPSEN (1938) would be obtained if the Kasauli antivenine (bi-valent anti-cobra and anti-daboia) was titrated in different animals and by different routes, and (2) to devise a suitable method for the standardization of a particular type of antivenine against the corresponding venom.

* The unusual spelling "venine"—which has the authority of the *Oxford English Dictionary*—has been retained.—ED

Standardization of dabova antivenom

The experimental work detailed herein was carried out for over 4 years, one particular sample of venom could not be used throughout. In the initial stages, the same sample of dabova venom which had been used for the routine titration of antivenomes for number of years was employed. It was kept in weighing bottle over potassium hydroxide in desiccator in the dark at room temperature. It was freshly made up for each experiment to the concentration required by dissolving in normal saline solution and was kept in the refrigerator until actually used. The same sample of concentrated antivenom kept in the refrigerator was generally used for each set of experiments to give comparable results.

Standard glassware was used throughout for making dilutions. The venom and antivenom were injected immediately after mixing.

Owing to the absence of previous data on the subject, a large number of animals had to be used for preliminary exploratory ranging of the amount of venom neutralized by 1 c.c. of concentrated antivenom. The final ranging figures were arrived at by the use of one or two large animals (monkey, rabbit) and two or three smaller animals (rice guinea-pigs, peccary, etc.) for each test.

A summary of the results is presented in Table I.

TABLE I
NEUTRALIZATION OF DABOVA VENOM BY 1-5 C.C. OF CONCENTRATED ANTIVENOM IN
DIFFERENT MICE DIFFERENT ROUTE

Animal	Weight (grams)	Route of injection	Total amount neutralized, c.c.	Lethal dose of venom c.c.	Net amount neutralized, m	Time of death after injection
1	2		3	5	6	7
Mouse	14-54	Intra-venous	0.75	0.01	1	min. -5 hr
Guinea-pig	565-644		1.0	0.01	1	3 min. -24 hr
	74-334		2.0	0.01		
	190-279	Subcutaneous	1-13	3.0	1	1 hr -48 hr
Rabbit	1,381-1,434	Intra-venous	1-9	0.2	1	2 min. -8 hr
	744-		2.9	4	1	
Monkey	1,644-2,304		10-17	2	12-14	2 hr -10 hr
	1,870-444	Subcutaneous	1-31	10	14-1	12 hr -36 hr
Peccary	741-220	Intra-venous	4-5	0.1	4	1 min. -1 hr
*Guinea-pig	744-751	Intraperitoneal	2-4	0.4	1.4	3 1/2 hr -7 hr
*White	1644-17		2	0	2	0 hr. -1 hr

Different batches of venom and antivenom used

(1) The venom is said to be neutralized (columns 4 and 6 in Table I), when all the animals survived the injection of mixed venom and antivenom.

The term 'Lethal dose' means the smallest amount which killed all the animals injected.

(2) The 'net amount neutralized' is arrived at by deducting the lethal dose from the total amount neutralized. This figure gives more accurate idea of the amount actually neutralized by 1 c.c. of antivenom alone without the assistance of the defence mechanism of the animal. In the case of the small animal where the lethal dose by the intra-venous route is comparatively very small (less than 0.4 mg.), the 'net amount neutralized' is taken to be the same as the 'total amount neutralized'.

(4) The lethal dose could be ascertained with a considerable degree of accuracy whether the injections were given by the intravenous, subcutaneous or intraperitoneal route except in the case of rabbits injected subcutaneously. Here the results were very irregular and have, therefore, been omitted.

(5) The time of death after injection of either the CLD or unneutralized venom-antivenine mixtures is indicated to show the advantage of using the intravascular route for injection.

It will be seen from the table that —

(1) Widely divergent amounts of the venom were neutralized in different species of animal by different routes and also in the same species of animal by different routes.

(2) The lethal dose was not in proportion to the weight of different animals.

It follows that, in the absence of a national or international standard, the name of the animal and the route of injection must be specified when the titre of any antivenine is recorded.

Neutralization by antivenine of daboia venom in multiples of a CLD

BANIC and LJUBETIC (1938) pointed out that the amount of serum required to neutralize 2 CLDs (certain lethal dose) is much larger than twice the amount of serum neutralizing 1 CLD. This lack of proportion was explained by assuming that a large part of 1 CLD is neutralized by the natural defence mechanism of the body, so that only a small portion of the injected venom requires neutralization by the serum to protect the animal. The actual quantity of serum required to neutralize a dose of only 1 CLD is arrived at by deducting the quantity neutralizing 1 CLD from the amount neutralizing 2 CLDs. These authors were of opinion that the higher multiples of 1 CLD of venom were neutralized by proportionate amounts of the antiserum. IPSEN (1938) first determined the MLD of the European viper venom and the effect of 0.25 c.c. of serum on varying amounts of higher multiples of 1 MLD. The results were represented graphically, the abscissa giving the amount of venom and the ordinate the quantity of serum. Mixtures containing four different amounts (0.05 c.c. to 0.2 c.c.) of serum and a suitable amount of venom chosen from the graph were injected intravenously into mice. A curve was again plotted and the neutralization of the venom by the antiserum at different levels was determined from the graph. If 0.25 c.c. of the serum neutralized 0.145 mg. venom and 0.15 c.c. neutralized 0.093 mg. of venom, then the potency of the serum was as follows:

$$\text{Titre} \quad \frac{0.145 - 0.093}{0.25 - 0.15} = \frac{0.052}{0.1} = 0.52$$

i.e., 1 c.c. neutralizes 0.52 mg. of venom.

IPSEN (1938) suggested that the assay of antivenine be performed at different levels and the relationship calculated between the dose of serum and the dose of venom which is completely neutralized. This formula can be applied only if there is a definite relationship between the amount of venom and the serum required to neutralize it irrespective of the level of test chosen.

This method, originally devised by BANIC and LJUBETIC (1938) and modified by IPSEN (1938), was employed in the titration of daboia antivenine.

The results are presented in Table II.

The calculated amount of serum neutralizing 1 CLD in pigeons by the intravenous route was 0.00105 c.c. (0.0015 c.c. protecting against 2 CLDs).

minus 0.0045 c.c. protecting against 1 CLD). In order to neutralize 4 CLD 0.0047 c.c. of serum should be required by calculation, whereas experimentally it was actually 0.0033 c.c. (slightly more than three times the amount required to neutralize 1 CLD). For 8 CLDs, the estimated amount of serum was 0.0094 whereas it was found experimentally to be 0.00855, a very close approximation. For 24 CLDs, the amount of serum theoretically needed was 24 times 1)

TABLE II.
TITRATION OF ANTIVENES MULTIPLES OF CLD DABOIA ANTIVENINE IN
QUANTITIES TO SERUMS INJECTED INTO VENOM.

Animal structures	Volume of serum, cc	Antivenine required.		CLDs	Proportionate amount of antivenine required.	
		Total c.	Net c.c.			
Guinea-pigs (200-230)	0.0	0.0000		1		
	0.01	0.00	0.00183			
	0.1	0.012	0.01183	5	0.0183	2.1
	0.2	0.02	0.01983	10	0.0183	11.3
	0.3	0.03	0.01983	15	0.0183	17.4
	1.0	0.08	0.08083	30	0.0183	30.4
Pigeons (200-230)	0.1	0.00413				
	0.02	0.0012	0.00181			
	0.05	0.00713	0.0025	5	0.0183	2.1
	0.1	0.01	0.00853	10	0.0183	9.1
	0.25	0.025	0.03183	15	0.0183	30
	3	0.04	0.08253	30	0.0183	75
	8	1	0.00257	60	0.0183	95
	0.5	0.14	0.135	75	0.0183	170
	1.25	0.3	0.2913	175	0.0183	225

amount required to neutralize 1 CLD whereas it was 30 times by actual experiment. There was no regular proportion between the dose of the venom and antivenine when 24 CLDs or more were used. In the case of guinea-pigs injected intravenously disproportionately more and more serum was required as the dose of venom was increased.

HAZRA, LAHRI and SARKAR (1945) claimed that by injecting mixtures of daboia venom and antivenine into mice by the intravenous route the relation between the antivenine and the corresponding venom could be determined irrespective of the level of the test chosen. They used only one mouse for the titration of each mixture. There being possibility that mice might respond differently to the action of daboia venom and antivenine mixtures the reaction of mice to the injection of the aforementioned mixtures was determined. The weight of mice used in different experiments varied from 18 to 25 grammes but mice of the same weight were used for titrating the neutralizing power of

a fixed amount of antivenine against varying doses of the venom. Most of the mixtures were injected after incubation at 37° C for 30 minutes, in a few experiments the mixtures were injected shortly after mixing. The results of injection of unincubated mixtures were not more irregular than in the case of incubated mixtures, a slightly bigger amount of venom was, however, neutralized by the antivenine after incubation. In view of the irregularity of the response of mice, the results of a number of experiments have been summed up in Table III.

TABLE III

NEUTRALIZATION BY DIFFERENT AMOUNTS OF ANTIVENINE OF VARYING MULTIPLES OF A CLD OF DABOIA VENOM IN MICE INJECTED INTRAVENOUSLY

Serum, c.c.	Venom, mg	* Mortality	Amount of venom neutralized by 1.0 c.c. of serum, mg	Serum, c.c.	Venom, mg	Mortality	Amount of venom neutralized by 1.0 c.c. of serum, mg
0.25	0.225	2/4		0.25	0.2	2/2	
0.25	0.20	0/4	0.8		0.18	1/2	
0.25	0.18	1/1			0.165	0/1	0.60
0.2	0.2	0/4	1.0	0.2	0.18	1/1	
0.2	0.18	0/2			0.165	0/1	0.8
0.15	0.18	0/1		0.15	0.2	0/1	
0.15	0.165	1/2	1.0		0.18	1/2	
0.15	0.15	0/1			0.165	0/2	1.1
0.1	0.125	3/4		0.1	0.14	1/1	
0.1	0.11	1/4	1.1		0.125	0/2	1.25
0.05	0.1	2/2		0.05	0.09	1/1	
0.05	0.09	1/4	1.8		0.075	0/1	1.5
0.025	0.07	1/2	2.4	0.025	0.055	1/1	
					0.045	0/1	1.8
Control 0.01 mg = 3/3 *Mortality = $\frac{\text{number of mice dead}}{\text{number of mice injected}}$ Mixtures incubated at 37° C for 30 min				0.25	0.2	4/4	
					0.18	3/4	
					0.165	1/2	0.6
				0.2	0.18	3/4	
					0.165	3/4	
					0.15	1/2	0.65
				0.15	0.18	0/2	
					0.165	1/4	1.2
					0.15	1/4	
				0.1	0.15	1/2	
					0.135	0/4	1.35
				0.05	0.1	2/2	
					0.085	0/2	1.7
				0.025	0.05	1/2	
					0.045	0/2	1.8

It can be concluded that it is not possible to determine exactly the titre of daboia antivenine by using just a few widely spaced dilutions and a single mouse per mixture. It has also been demonstrated that with the gradual increase in the amount of antiserum in the mixture, proportionately less and less of venom is neutralized.

There is thus definite evidence that the method of BANC and LJSZTIC or IRSEN is not suitable for the titration of daboia antivenine because (1) multiple CLDs of venom are not neutralized by proportionate multiples of antiserum and therefore the exact titre of the antivenine cannot be determined by this method. (2) a very large number of animals would be required for each titration.

Standardization of Cobra Antivenine

As in the case of daboia venom no detailed work has been carried out on the neutralization of cobra venom by its antivenine in different animals by different routes and in the same animal by different routes. Experiments were, therefore, carried out by titrating generally the same sample of antivenine against the same batch of cobra venom. Standard glassware was used for making the dilutions. The venom-antivenine mixtures were incubated at 37° C. for 30 minutes before injection.

A large number of animals were used to arrive at a preliminary figure in respect of the MLD and the amount of venom neutralized by 0.25 c.c. of antivenine. The final ranging was carried out by using one or two large animals (monkey, rabbit) and two or three smaller animals (mouse, guinea-pig, pigeon etc.).

The results summarized in Table IV show that the net amount of venom neutralized by 0.25 c.c. of concentrated antivenine injected by different routes in different animal varies markedly. Titration in the same animal by different routes also gives variable results. These differences are, however, not so marked as in the case of daboia venom.

NEUTRALIZATION BY ANTIVENINE OF COBRA VENOM IN MULTIPLES OF A CLD

TAYLOR (1940) reported confirmation of the work of IRSEN (1938) by titrating cobra venom in mice by the intramuscular route but he found a correlation between the amount of venom and antiserum up to 5 CLDs only. When he used 10 CLDs, he had to use more serum than the estimated amount. CALMETTE (1906) had reported lack of proportion between the amount of cobra venom and the antivenine required when injecting mice by the subcutaneous route. It is possible that had TAYLOR (1940) tried more than 10 CLDs there might have been an appreciable disproportion between the amounts of venom and antivenine.

TAYLOR's experiments have been repeated with certain modifications. His

TABLE IV
TITRATION OF COBRA VENOM IN DIFFERENT LABORATORY ANIMALS BY DIFFERENT ROUTES

Animal 1	Weight grammes 2	Route of injection 3	0.25 c.c. of antivenine - varying amounts of venom			
			Total amount neutralized, mg 4	MLD, mg 5	Net amount neutral- ized, mg 6	Time of deaths 7
Rabbit	1,240	Intravenous	0.6	0.2	0.4	3 min - 64 min
	2,180	Intramuscular	0.7	0.4	0.3	
Pigeon	1,700-1,800	Intraperitoneal	0.9	0.7	0.2	76 min - 3½ hr
	300	Intravenous	1.0	0.5	0.5	
Guinea pig	400-500	"	0.4	0.05	0.35	1 hr - 5 hr
	300	Intramuscular	0.5	0.2	0.3	
	300	"	0.7-0.8	0.3	0.4-0.5	15 min - 2½ hr
		Intravenous	0.35	0.15	0.2	
		Subcutaneous	0.3	0.07	0.23	18 hr - 25 hr
		Intraperitoneal	0.4	0.1	0.3	
	500-540	Intravenous	0.4	0.1	0.3	45 min - 7½ hr
		Intraperitoneal	0.4	0.15	0.25	
Mouse		Intravenous	0.4	0.3	0.2	30 min - 4½ hr
		Intraperitoneal	0.5	0.01	0.20	
* Monkeys	2,000-2,600	Intravenous	1.0	0.5	0.5	1 hr - 2 hr
White rat	1,800-2,000	Subcutaneous	3.0	2.0	1.0	
	110-120	Intraperitoneal	0.7	0.08	0.02	3 hr - 6 hr
						6 hr - 48 hr

* Different batch of venom and antivenine used

employed the intramuscular route of injection in mice and could use only 0.2 c.c. of the mixture, while in these experiments 2.0 c.c. of the mixture was injected into mice by the intraperitoneal route. The venom-antivenine mixtures were injected without any incubation. A very large number of mixtures containing different proportions of venom and antivenine had to be prepared and each mixture was injected into one mouse only, but by employing a large number of different dilutions of antivenine at closely spaced intervals, fairly regular results were obtained.

The results are shown in Table V. It appears from the data given in Table V that more or less proportionate amounts of antivenine are required to neutralize multiples of CLD of cobra venom. The maximum amount of venom used in these experiments was only 0.25 mg. In the next series of experiments detailed in Table VI, the amount

TABLE V

NEUTRALIZATION OF MIXTURES OF COBRA VENOM IN MIXTURE INJECTED INTRAVENOUSLY

Amount of venom mg.	Amount of antivenine (c.c.) required		Number of C.I.D.s	Proportionate amount of antivenine required.
	Total.	Net.		
0.01	0.0008		1	
0.02	0.012	0.0111	2	
0.03	0.013	0.0141	3	0.0111 1.3
0.04	0.0178	0.027	4	0.0135 3.4
0.05	0.043	0.0441	5	0.0111 4.0
0.1	0.1	0.0991	10	0.0111 6.6
0.25	0.2	0.191	25	0.0111 22

of venom neutralized by 0.25 c.c. and 1.0 c.c. of antivenine in different animals was determined. A definite lack of proportion between the amount of venom and antivenine was observed when different amounts of antivenine were used in the mixtures injected. It is thus clear that the suggestion of IMRAY (1933) to

TABLE VI

NEUTRALIZATION OF COBRA VENOM AND 0.25 C.C. AND 1.0 C.C. OF ANTIVENINE IN MIXTURES

Animal	Weight, grammes	Route of injection	Quantity of serum	Total amount neutralized, mg.	M.L.D. mg.	Net amount neutralized per c.c. of serum, mg.
Rabbit	1.700	Intravenous	1.0	1.5	0	1.5
	1.40		0.25	0.8	0	2
	1.700-1.600	Intramuscular	1.0	2	0.7	1.3
			0.25	0.8	0.7	2.0
Pigeon	400-300	Intravenous	1.0	1.8	0	1.8
			0.5	0.5	0.2	1.3
Guinea-pig	300-34		1.0	1.2	0.15	1.05
			0.5	0.4	0.15	1.45

carry out the assay at different levels and thereby determine the proportion of venom and antivenine required for complete neutralization is not strictly applicable to the titration of cobra antivenine.

Remarks on the Standardization of Antivenine

Owing to the multiplicity of venoms and antivenines and the practice of preparing polyvalent antivenines the preparation and use of standard antivenines is not convenient but desirable. It would be easier to state the potency

of antivenine in terms of the number of CLDs of the venom neutralized by a stated amount of antivenine. For this purpose it would be advisable to select a particular batch of venom dried to constant weight and to determine its CLD in a suitable experimental animal injected by a specified route. Among the usual laboratory animals, rabbits are expensive and require fairly large doses of venoms and antivenines. The suitability of pigeons bought from dealers and guineapigs and mice bred in the laboratory was compared from the point of view of variability in their resistance. The results are presented in Tables VII and VIII.

It was found that an exact titration of antivenines in pigeons (not in-bred) could be carried out only at 30 per cent intervals, whereas an intracardial titration in guineapigs could be accurately carried out at 10 per cent intervals. The titration could also be carried out in guineapigs by the intramuscular or

TABLE VII
TITRATION OF COBRA ANTIVENINE IN GUINEAPIGS AND PIGEONS
0.25 C.C. ANTIVENINE + VARYING AMOUNTS OF COBRA VENOM

Pigeons				Guineapigs			
Weight, grammes	Route of injection	Dose of venom, mg	Mortality rate	Weight, grammes	Route of injection	Dose of venom, mg	Mortality rate
230-250	Intravenous	0.1	0/3	230-250	Intracardial	0.45	0/3
		0.55	1/1 D* 47 min			0.5	0/3
		0.60	2/3 D 33-55 min			0.55	2/3 D 1½-2 hr
200-220	Intravenous	0.6	0/4	240-270	Intracardial	0.45	0/3
		0.66	3/4 15 min -1½ hr			0.5	1/3 D 8 hr
		0.72	3/4 26 min -1 hr			0.55	3/3 D 4½-9 hr
		0.8	4/4 15-33 min			0.6	3/3 D 2½-3 hr
200-220	Intravenous	0.9	0/1	290-320	Intracardial	0.45	0/3
		1.0	1/5 D 1½ hr			0.5	0/3
		1.1	3/5 D 45-70 min			0.55	3/3 D 1½-5½ hr
200-220	Intramuscular	1.2	1/4 D 14 hr			0.6	4/4 D 1½-1½ hr
		1.4	3/4 D 7-21½ hr	290-320	Intramuscular	0.9	0/3
		1.6	3/4 D 13½-1½ hr			1.0	3/3 D 13-36 hr
200-220	Intracardial	1.6	0/1			1.1	3/3 D 12-16 hr
		1.8	2/4 D 9-13 hr	240-260	Intraperitoneal	0.8	0/3
		2.0	4/4 D 8½-11 hr			0.9	3/3 D 7-11½ hr
						1.0	3/3 D 3-9 hr
				290-320	Intravenous	0.6	0/3
						0.66	4/4 D 1-2 hr
						0.72	4/4 57-67 min

* D = Dead

TABLE V

NEUTRALIZATION OF MULTIPLE CLDS OF COBR. VENOM IN MIXT. 1 INJECTED INTRAPERITONEALLY

Amount of venom, mg.	Amount of antivenine (c.c.) required		Number of CLDs.	Proportionate amount of antivenine required.	
	Total.	Net			
0.01	0.0000		1		
0.02	0.012	0.0111	2		
0.03	0.013	0.0111	3	0.0111	1.3
0.04	0.0178	0.017	4	0.0111	3.4
0.05	0.013	0.0111	5	0.0111	4.0
0.1	0.1	0.0991	10	0.0111	0.0
0.25	1.3	0.191	25	0.0111	22

of venom neutralized by 0.25 c.c. and 1.0 c.c. of antivenine in different animals was determined. A definite lack of proportion between the amount of venom and antivenine was observed when different amounts of antivenine were used in the mixtures injected. It is thus clear that the suggestion of LEECH (1938):

TABLE VI

NEUTRALIZATION OF COBR. VENOM BY 0.25 c.c. 1.0 c.c. OF TITRATED ANTIVENINE

Animal	Weight, grammes	Route of injection.	Quantity of serum.	Total amount neutralized, mg.	M.L.D. mg.	Net amount neutralized per c.c. of serum, mg.
Rabbit	1,700	Intra-venous	1.0	1.0	0	1.3
			0.25	0.0	0	2
	1,700-1,800	Intramuscular	1.0	2	0.7	1.8
			0.25	0.0	0.7	2.0
Pigeon	400-500	Intr. venous	1.0	1.0	0.3	1.4
			0.25	0.5	0.2	1.8
Guinea-pig	300-350		1.0	1.2	0.15	1.05
			0.25	0.4	0.15	1.45

carry out the assay at different levels and thereby determine the proportion of venom and antivenine required for complete neutralization is not strictly applicable to the titration of cobra antivenine.

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Owing to the multiplicity of venoms and antivenines and the practice of preparing polyvalent antivenines, the preparation and issue of standard antivenines is not convenient but desirable. It would be easier to state the potency

of antivenine in terms of the number of CLDs of the venom neutralized by a stated amount of antivenine. For this purpose it would be advisable to select a particular batch of venom dried to constant weight and to determine its CLD in a suitable experimental animal injected by a specified route. Among the usual laboratory animals, rabbits are expensive and require fairly large doses of venoms and antivenines. The suitability of pigeons bought from dealers and guineapigs and mice bred in the laboratory was compared from the point of view of variability in their resistance. The results are presented in Tables VII and VIII.

It was found that an exact titration of antivenines in pigeons (not in-bred) could be carried out only at 30 per cent intervals, whereas an intracardial titration in guineapigs could be accurately carried out at 10 per cent intervals. The titration could also be carried out in guineapigs by the intramuscular or

TABLE VII
TITRATION OF COBRA ANTIVENINE IN GUINEAPIGS AND PIGEONS
0.25 C.C. ANTIVENINE + VARYING AMOUNTS OF COBRA VENOM

Pigeons				Guineapigs			
Weight grammes	Route of injection	Dose of venom, mg	Mortality rate	Weight, grammes	Route of injection	Dose of venom, mg	Mortality rate
230-250	Intravenous	0.5	0/3	230-250	Intracardial	0.45	0/3
		0.55	1/3 D* 47 min			0.5	0/3
		0.60	2/3 D 33-55 min			0.55	2/3 D 1½-2 hr
290-320	Intravenous	0.6	0/4	240-270	Intracardial	0.45	0/3
		0.66	3/4 15 min -1½ hr			0.5	1/3 D 8 hr
		0.72	3/4 26 min -1 hr			0.55	3/3 D 4½-9 hr
290-320	Intravenous	0.8	4/4 15-33 min		Intracardial	0.6	3/3 D 2½-3 hr
		0.9	0/5	290-320		0.45	0/3
		1.0	1/5 D 1½ hr			0.5	0/3
		1.1	3/5 D 45-50 min			0.55	3/3 D 1½-5½ hr
290-320	Intra- muscular	1.2	1/4 D 14 hr	290-320	Intra- muscular	0.6	4/4 D 1¼-1½ hr
		1.4	3/4 D 7-21½ hr			0.9	0/3
		1.6	3/4 D 13½-15 hr			1.0	3/3 D 13-36 hr
290-320	Intra- muscular	1.6	0/4	240-260	Intra- peritoneal	1.1	3/3 D 12-16 hr
		1.8	2/4 D 9-13 hr			0.9	0/3
		2.0	4/4 D 8½-11 hr			0.9	3/3 D 7-11½ hr
				290-320	Intravenous	1.0	3/3 D 3-9 hr
						0.6	0/3
						0.66	4/4 D 1-2 hr
						0.72	4/4 57-67 min

* D = Dead

intraperitoneal routes, but it took much longer to read the results. When the titre of antivenines was determined by the intracardial and intravenous routes of injection in guineapigs, the results were identical. The latter route, being cumbersome in guineapigs, was discarded. Mice were found to be quite suitable from the point of view of their uniform resistance but one can inject only relatively small doses intravenously and to ensure greater accuracy in titration one should be able to inject at least 2.0 c.c. of mixtures into test animal. Moreover mice are generally difficult to breed in the plains in India except in air-conditioned rooms, while guineapigs breed readily both in the hill and the plains.

TABLE VIII

TITRATION OF CORBA ANTIVENINE IN MICE OF DIFFERENT WEIGHTS
OF ANTIVENINE + ARTIFICIAL MIXTURES OF VENOM INJECTED INTRAVENOUSLY

Weight, grammes.	Dose of venom, ccg	Mortality rat	Time of death.
20-25	0.1	3	
	0.17	2.3	20-25 min.
	0.2	2.3	14-14 min.
10-12	0.15	0.3	
	0.21	1.3	3 min.
	0.4	3.3	13 min. 1 hr
14-21	0.21	0.3	
	0.23	1.3	87 min.
14-1	1	0.3	
	1.3	1.3	1 1/2 hr

It is thus clear that in India, at any rate guineapigs are most suitable for the titration of antivenines. It was found in the preceding experiments that guineapigs weighing either from 230 to 260 grammes or 290 to 320 grammes could be used. As it is not always possible to obtain an animal of the required weight experiments were conducted to determine whether guineapigs of different weights could be used. The results for cobra venom are presented in Table IX.

There was only 10 per cent difference in titre due to variation in the weight of the guineapigs from 260 to 470 grammes. It would be advisable to use animals in one particular range of weight, 290 to 320 grammes, for exact titration, but lighter or heavier guineapigs could be used for the preliminary determination of potency of unknown sera if animals of standard weight were not available in large numbers. These remarks hold good for daboya venom also.

Determination of the titre of antivenine in horses under immunisation with cobra and daboya venoms

There were 17 horses under immunisation. They were bled at intervals during the course of injections with mixtures of cobra and daboya venoms. Mixtures were prepared containing 1 of 1.4 dilution of these sera along with varying amounts of each of the

test venoms and the mixtures were injected without incubation intracardially into guinea-pigs weighing 290 to 320 grammes, 156 tests were carried out with both venoms. Sera from 15 of these horses neutralized about 2 CLDs of cobra and daboia venoms. If the amount of serum neutralizing 1 CLD of venom is considered to contain 1 unit, then these natural sera had about 8 units of antivenine per c.c.

TABLE IX

TITRATION OF COBRA ANTIVENINE IN GUINEAPIGS OF DIFFERENT WEIGHTS
0.25 c.c. OF ANTIVENINE + VARYING AMOUNTS OF VENOM INJECTED INTRAVENOUSLY

Weight, grammes	Dose of venom mg	Mortality rate	Time of death
240-250	0.35	0/3	5½-12 hr
	0.40	0/3	
	0.45	3/3	
290-320	0.35	0/3	
	0.40	0/3	
	0.45	1/3	
380	0.4	0/3	9 hr
	0.45	0/3	
	0.5	2/2	
450-470	0.45	0/3	11-14 hr
	0.50	3/3	10-11 hr

Forty concentrates prepared from these sera were found to contain at least 32 units (1 c.c. of a 1/16 dilution of the concentrate neutralized at least 2 CLD of venom). Natural sera from the remaining two horses contained about four units of antivenine per c.c.

Potency of Antivenine Prepared by Another Reputable Manufacturer

Samples of quadrivalent antivenine prepared by another well-known Institute were examined with regard to their anti-cobra and anti-daboia potencies. One c.c. of a 1/16 dilution of the concentrate neutralized slightly less than 2 CLDs of venom.

A deterioration of at least 10 per cent per annum has to be allowed for during storage of antivenines. It is, therefore, legitimate to expect a minimum standard of 16 units per c.c. in the case of anti-cobra and anti-daboia sera, i.e., 1 c.c. of a 1/16 dilution of the concentrate must neutralize at least 1 CLD of venom.

Advisability of Determining the CLD of a Test Venom

It is the practice in a number of laboratories to indicate the potency of an antiserum in terms of mg. of venom neutralized, but different batches of cobra and daboia venoms differ appreciably in potency. A large number of experiments were carried out on the subject and it was found, for example, that one batch of cobra venom had a CLD of 0.07 mg., whilst in another the CLD was 0.18 mg. Similarly, one batch of daboia venom was thrice as lethal as another

It is thus clearly inadvisable to indicate the potency of an antiserum in terms of mg. of venom neutralized.

Incubation of venom + antivenine mixtures.

In preliminary series of experiments, venom + antivenine mixtures were injected intracardially into guinea-pigs (a) immediately after mixing and (b) after an incubation of 30 minutes at 37°C. It was found that, in general, neutralization of the venom improved by about 10 per cent. after incubation, but the results of injection of these mixtures were quite regular. The incubation of venom + antivenine mixtures, consequently is not considered necessary.

Method of standardization recommended

- (1) The test venom should be dried to constant weight and kept in the cold in an evacuated desiccator over suitable drying-agent e.g., phosphorus pentoxide.
- (2) The test venom should be weighed immediately before use and dissolved in the requisite volume of normal saline solution.
- (3) As there is no definite proportion between the amount of serum and the dose of venom neutralized at different levels of test, the antivenine under test, being usually concentrate, should be diluted 1:16 and mixed in the proportion of 1.0 c.c. of diluted antivenine and 1 to 2 CLDs of the test venom.
- (4) A mixture for four animals should be prepared and made up to 8 c.c. with normal saline solution, 2 c.c. to be injected intracardially into each of three guinea-pigs weighing 250 to 320 grammes before food.
- (5) Incubation of venom + antivenine mixtures is not necessary.
- (6) The maximum period of observation of the injected animals should be 24 hours, any guinea-pig surviving up to this period being recorded as alive; death after 24 hours is very uncommon.
- (7) A reasonable standard suggested for adoption is that 1 c.c. of 1:16 dilution of concentrated antivenine should neutralize at least 1 CLD of the venom.

SUMMARY

Widely divergent amounts of test venoms are neutralized by the same amounts of their corresponding antivenines when the mixtures are injected into different animals by different routes or in the same animal by different routes.

There is no definite proportion between the quantity of antivenine and the amount of venom neutralized at different levels of test.

Different batches of cobra and dabola venoms differ appreciably in potency. It is therefore inadvisable to indicate the titre of an antivenine in terms of mg. of venom neutralized. It should be stated in terms of the number of CLDs neutralized.

A suitable method of titration of cobra and dabola antivenines has been described.

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THE EXTERMINATION OF *ANOPHELES GAMBIAE* IN THE WADI HALFA AREA *

BY

D J LEWIS, M A ,

Medical Entomologist, Stack Medical Research Laboratories, Khartoum

The mosquito fauna of the Wadi Halfa area and the measures for the control of *Anopheles gambiae* used in 1943 were described by LEWIS (1944). The northern limit of this tropical anopheline was probably for many years in the Aswan Reservoir area, most of which lies within the tropics. In 1942 and 1943 a very serious epidemic of malaria occurred in Egypt north of Aswan, as a result of which the authorities in Egypt undertook a campaign to exterminate *A. gambiae* in that country (FARID, 1943, MACDONALD, 1946, Rockefeller Foundation, 1946, WORTHINGTON, 1946). In 1944 and 1945 control measures were intensified in the Wadi Halfa area and extended into the Second Cataract in order both to minimize the chance of *A. gambiae* spreading to Egypt, thus hindering the extermination campaign, and to prepare the way for extermination in the Wadi Halfa area when the campaign in Egypt should prove successful. *A. gambiae* was exterminated in Egypt in 1945, and accordingly plans for extermination in the Wadi Halfa area were put into operation in the winter of 1945 to 1946. They were successful, and at least 90 km of river are now free of *A. gambiae*, including irrigated areas and the numerous islands and channels of the Second Cataract. This stretch of river is not comparable to the large area of Brazil cleared of *A. gambiae* (SOPER and WILSON, 1943) and is far smaller than the extermination area in Egypt. The Wadi Halfa extermination is of interest, however, because, as far as is known, it is the first instance of the extermination of this species in an area of the Ethiopian zoogeographical region where it has occurred for many years, and because the work proved very easy and only required the use of little more than the normal staff.

* The writer is very much indebted for information on certain points to Dr MUHAMMAD AHMED ALI, M B E, and SAYED EFFENDI MUHAMMAD MAHMUD, Medical Inspector and Public Health Officer of the district who were responsible for carrying out the work of extermination, and to his assistant AHMED EFFENDI ABDEL RAHMAN BEREIR.

Prior to extermination routine control had made *A. gambiae* uncommon. It was occasionally found in the Cataract until the last specimens were obtained in June 1945. No *A. gambiae* have been found between Wadi Halfa and Faras since larvae were taken at Wadi Halfa on 19th December 1943.

This paper contains an account of the work of extermination, and, owing to the importance of the area, general information is included on *A. gambiae* and other mosquitoes in the Wadi Halfa area and districts to the south.

The information was obtained during several visits by the writer and from routine reports and specimens received for identification.

An abstract in the *Journal of Tropical Medicine* (50: 38) states that in 1942 *A. gambiae* spread from Central Africa into Egypt, and WORTHINGTON (1946) refers to suggestions that the northward spread of *A. gambiae* may have been associated with air transport from West and Central Africa. In the present writer's opinion, there is no evidence that *A. gambiae* spread from Central Africa and every probability that it spread from the Aswan Reservoir area. The northern limit of *A. gambiae* before 1942 is not known, but it is of interest to note that it was found at Merowe, 18° 29' N. in January 1938, and was recorded near Merowe or even further north as long ago as the winter of 1908 to 1907 (LEWIS, 1945). Reasons given by LEWIS (1944) suggest that *A. gambiae* has occurred in the Wadi Halfa area for many years, and it seems probable that this Ethiopian mosquito has occurred in, or visited the Aswan Reservoir area, which lies mainly in the tropics, for a long period. WORTHINGTON (1946) refers to the possibility of the previous occurrence of *A. gambiae* in Egypt. In the Annual Report of the Sudan Medical Service for 1936 a difficult problem of malaria control at Faras was reported to be due to seepage from the Aswan Reservoir. *A. gambiae* may be suspected to have occurred, although *A. walkeri* was probably present. The report for 1929 mentions malaria at Wadi Halfa. A severe outbreak of malaria in the reservoir area north of Faras in 1919 (Reports, etc., 1920, page 65; 1921, page 88) can probably be regarded as an indication of the presence of *A. gambiae* at that time since other vectors are unlikely to have been important in the narrow riverain area.

METHODS USED FOR EXTERMINATION

It was known that *A. gambiae*, even if uncontrolled, became very scarce in the winter but could breed at this time in the Second Cataract. The method adopted was simply to treat all probable breeding places between Faras and the north end of the Cataract with pyrethrum dust once a week from November to March regardless of whether larvae were found or not, normal work being continued north of the Cataract. The larvicide was prepared by the very simple and practical method described by BORER and WILSON (1943) being mixed with local dust in a paul at the breeding place. This work was done by 17 "mosquito men" under the supervision of one sanitary overseer and the part-time supervision of public health officer. Mosquito men are trained to recognise mosquito larvae and apply larvicides and are paid little more than unskilled labourers. Eleven worked in the Cataract, using ferries and hired boats to reach the island, and two worked to the south and four to the north of the Cataract. It is not unlikely that the eleven men in the Cataract area would have sufficed to achieve extermination by tackling the mosquito

in its winter focus at a season most unfavourable to it DDT was to be substituted for paris green, as it would have obviated the necessity of mixing larvicide on the spot, but the extermination of *A. gambiae* rendered it unnecessary. Although the work of extermination was a great credit to the staff concerned the official in charge readily admitted that it was very easy.

No control of adults was carried out apart from spraying of vehicles to prevent reinfestation and spraying houses near the railway station with the same object.

A simple monthly report of the work was sent to the Director of the Sudan Medical Service.

More extensive measures would, of course, have been used and more staff employed if *A. gambiae* had not been exterminated so easily.

Proof of Extermination

On 1st April, 1946, all anti-larval work was stopped north of Gemai, apart from the control of biting culicines at Faras. Numerous potential breeding places were formed as usual with the fall of the water level and rise of temperature. No *A. gambiae* appeared and the species has not been found in the Saras-Faras area since June, 1945.

Additional evidence of the complete extermination of *A. gambiae* was afforded by its failure to reappear in spite of the opportunities for breeding created by the great Nile flood of 1946, one of the highest ever recorded and probably the highest in the district for over a century. In the Wadi Halfa area some 14,000 acres of land were inundated and 10,000 people rendered homeless, 2,800 houses collapsed along the 25 km stretch of river from Halfa Degheim to Debeira. The temperature was favourable to breeding and, as the water level slowly fell, numerous pools appeared among the ruined houses (Figs 3 and 4), in pits made during the construction of flood-protection banks and in old borrow pits which are not filled because the absence of rain makes levelling unnecessary. Irrigation channels and areas of flooded grass formed other potential breeding places. Large numbers of *Culex univittatus* and some *Aedes caspius* bred, the latter having probably spread from Faras. The outbreak of culicines made *A. gambiae* conspicuous by its absence, particularly since it usually breeds in association with *C. univittatus*. Some residents, unaware of the absence of anophelines, took prophylactic atabrin.

Many breeding places were oiled between 19th September and 10th November, 1946, to control culicines, but this is not considered to have invalidated the test of extermination.

The Saras-Gemai area was treated with larvicide till February, 1947, but no anophelines were found in it for over a year. It is now untreated and free of *A. gambiae*. At the time of writing (December, 1947) 30 months have elapsed since *A. gambiae* was last found north of Saras, 21 since the test of extermination began, and 13 since the localized oiling necessitated by the flood.

It is possible that extermination was effected by natural causes but very improbable, since *A. gambiae* was present each year from 1941 to 1945 and had occurred there in previous years (LEWIS, 1944).

The Effects of Extermination.

A. gambiae is now absent from the 80 km. stretch of river from Saras to Faras, and at present is not known to occur further north than Ferka, where it was found in April 1947.

1



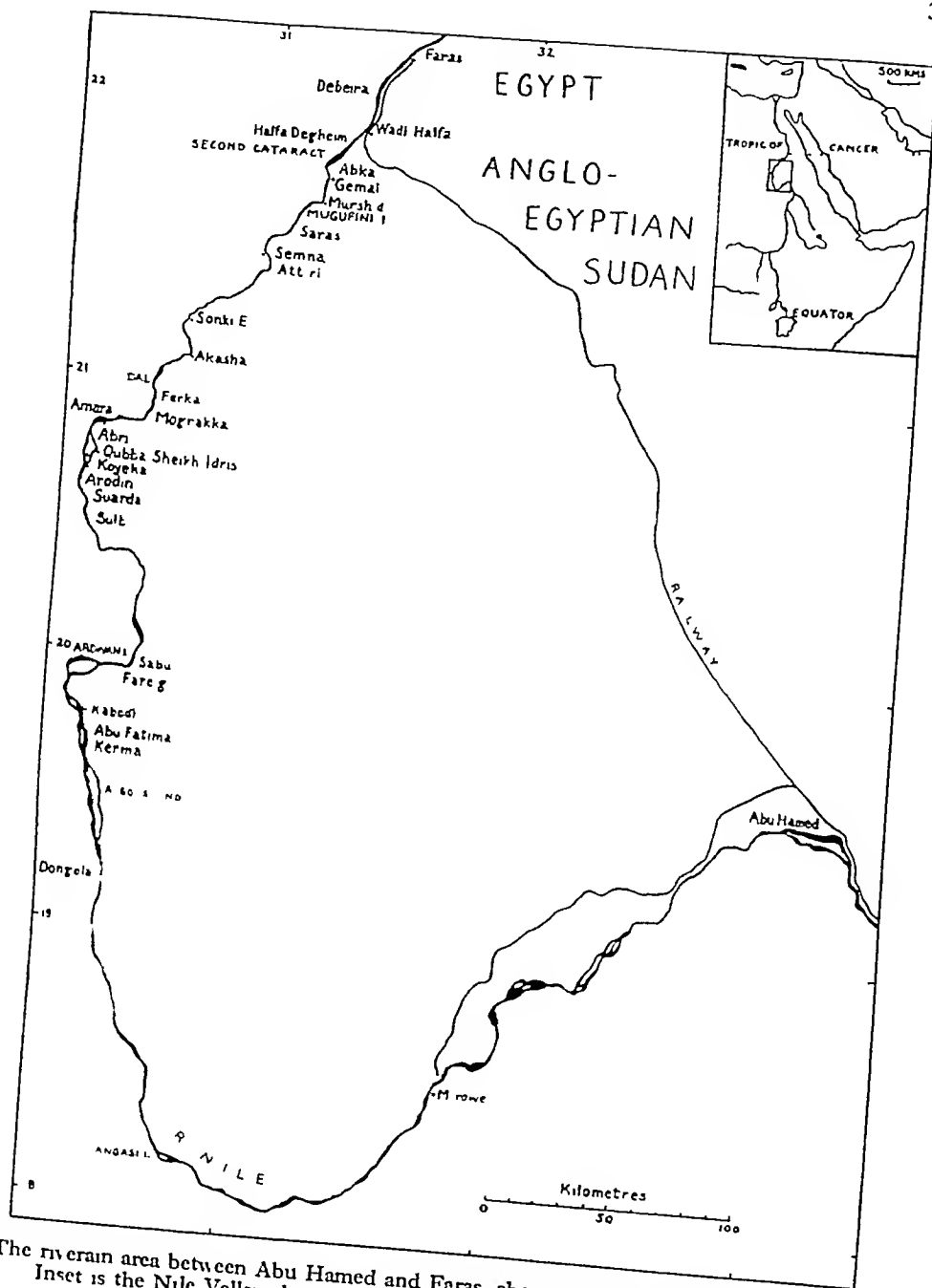
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FIG. 1.—Breeding places of *A. gambiae* near Debera in 1941.

FIG. 2.—The Nile near Saras: the river flows in deep narrow channel through the desert and the area forms natural barrier to northward spread of *A. gambiae*.

FIGS. 3 AND 4.—Pools left by the great Nile flood among ruined houses at Wadi Halfa in September 1946, after the extermination of *A. gambiae*: thousands of pools, devoid of anopheline larvae demonstrated the absence of this species.



The river area between Abu Hamed and Faras, showing places mentioned in the text
 Inset is the Nile Valley showing the position of the area

It is impossible for *A. gambiae* to enter Egypt from Wadi Halfa unless Wadi Halfa is re-invaded.

The Wadi Halfa area is free of malaria because no anophelines exist apart from a few individual of *A. multicolor* which occasionally appear at the remote Faras basin, 32 km. from Wad Halfa, where this species probably spreads from the north.

By a fortunate coincidence the year chosen for extermination proved to be that of the great Nile flood, so that the work prevented an epidemic of malaria from adding to the difficulties of the largely homeless population. An epidemic occurred further south in a comparable area upstream from Merowe, where an irrigated area is situated near a series of rapids. If *A. gambiae* had multiplied in the Wadi Halfa floods it might have spread to Egypt.

Extermination has resulted in the release of staff and equipment for use in other areas.

The Nile Between Merowe and Sarai.

This 356 km. length of river upstream of Sarai is outside the present area of extermination but may be briefly considered as it is a possible source of reinfestation and because the question of extending extermination southward should be considered.

The area has been described by many writers, notably BURKHARDT HUNT LYONS and WILLCOCKS and CRAIG, whose works are listed by HILL (1909). From Merowe to Kerma the Nile is navigable and flows largely between steep alluvial banks without forming numerous residual pools at low water. From Kerma to Sarai it passes through a series of rapids separated by calm stretches in a valley of rocky or sandy desert. Numerous pools form among the islands and mud banks in the rapids and other shallow areas as the water level falls between September and June. LONGFIELD (1935) describes the northern section as follows. For 100 miles south of Halfa the Nile flows through a narrow valley known to the Arabs as the *Bah el Hagar* or Belly of Rocks. The ground on either bank falls steeply and often precipitously to the water's edge and is scarred by ravines and broken by outcrops of granite. The current of the river is rapid and its course is impeded by innumerable reefs and rocky islets. The Merowe-Sarai area thus tends to afford numerous breeding places of *A. gambiae* in some places, and few or none in others. The 77 km. stretch of river between Akasha and Sarai is almost entirely inaccessible by road and consists largely of a deep narrow rocky gorge without pools. For great distances the river banks in the Kerma-Sarai area can only be reached by desert tracks which cross large areas of soft sand and boulder-strewn desert.

A. gambiae has been found at Abri, Akasha (January 1944), Arodin, Ardwan I land, Deiza, Ferka, Qubba Sheikh Idri, Kabodi, Koyeka, Merowe, Mograka, Sabu, S. elia and Tangari Island. As may be expected near the northern limit

of its range, in the country described above which is virtually rainless and subject both to occasional winter frosts and to very hot summers, *A. gambiae* appears to exist permanently in a series of isolated places and to extend its range in favourable months and years. In February, 1947, the writer examined pools in many places between Kerma and Saras and found *A. gambiae* only in a few widely separated places. The anopheline was apparently spreading from its centres of dispersal.

Malaria has a sporadic incidence as might be expected from the conditions under which *A. gambiae* lives and is said to break out from time to time in villages where it has not been known for years. The Annual Reports of the Sudan Medical Service for the years 1925 to 1943 indicate that malaria is most prevalent from December to April when the river is low, that there are occasional bad years when the river level falls irregularly, and that malaria is always prevalent in the islands north of Dongola. Between Abu Fatima and Saras outbreaks of malaria from time to time have necessitated the control of anophelines at Abri, Akashli, Atiri, Dil, Delgo, Ferka, Freig, Semna and Suurda.

PRESSENT AND FUTURE WORK

The Saras to Faras Area—This area is regarded as a barrier to prevent *A. gambiae* from spreading into Egypt and as a possible indicator of any risk of such a spread. It is a barrier since it is free of *A. gambiae* and could be freed again if necessary. It is an indicator because regular inspections should reveal the presence of *A. gambiae*, if it returned, long before it became numerous enough to reach Egypt.

No larvicidal measures are carried out except at Faras, and occasionally against biting culicines elsewhere. Between Saras and Gemai the river flows for 30 km through a narrow gorge containing only a few pools at widely separated points. This gorge is likely to act as a natural barrier to the northward spread of *A. gambiae* and is much longer than the 6 km larvicidal barrier which sufficed to prevent the spread of *A. gambiae* in a thickly populated area near Wadi Halfa (Lewis, 1944). In the area between Saras and Faras anti-larval measures are not only unnecessary now but they might mask a return of *A. gambiae* which would otherwise be rapidly detected by the regular inspection of pools at Murshid and Mugufini Island and further north. Each part to the area is inspected several times a month and a monthly report is sent to the Director of the Sudan Medical Service.

Trains from the south are sprayed with pyrethrum spray before reaching Wadi Halfa, and are treated with DDT residual spray. Trains are also sprayed at Abu Hamed from April to June, the period when breeding conditions are suitable for *A. gambiae* at Wadi Halfa and when trains often go beyond Wadi Halfa to Faras because steamers sail from this point if the river level is too low at Wadi Halfa. Aircraft are sprayed at Wadi Halfa and at southern aerodromes where

A. gambiae is under control (Ministry of Health Monthly Bull. 1944). It may be noted that WHITFIELD (1939), in a 3-years' collection of insects in aircraft at Khartoum, found 18 anophelines of which two were *A. gambiae* and ten undentifiable. It is considered unnecessary to treat the few motor cars which traverse the two long desert tracks from the south. Movement of boats through the cataracts is negligible except when, each autumn, the "date fleet" (REITER, 1929) sets out from Dongola for Abka. It is proposed to inspect the boats at Saras. Larvicidal equipment is kept always available.

The Saras-Faras barrier is additional to that maintained by the Egyptian authorities between Faras and Aswan. If ever *A. gambiae* should spread north of Faras, by natural means or in steamers, it would enter the long barrier area of the Aswan Reservoir. The Egyptian and Sudan authorities regularly exchange information on preventive measures.

It is not known if the Aswan Dam will ever be raised above its present height, but WORTHINGTON (1946) points out that a further rise of 15 metres would submerge Wad Halfa and bring the Reservoir level to the top of the Second Cataract. At present the upper half of the Cataract is unaffected and pools form during the storage period. The increased level in the district generally might bring about conditions suitable for the breeding of *A. gambiae* such as those described by FARM (1943) at Qena in Egypt.

GREYER (1945) has studied seepage problems, a subject which might prove of importance in the future.

The Merowe to Saras Area. *A. gambiae* is controlled by anti-larval measures at several places. This work reduces the chances of reinfestation of the Wad Halfa area and furnishes information on the northern distribution of the species. It is hoped that the Akasha-Saras area will, from its nature, act as a natural barrier to the northward spread of *A. gambiae* in addition to the natural Saras-Gemau barrier.

Following the work of extermination north of Saras the question naturally arises as to whether the work should be extended southward. SOFKA and WILSON (1943) have, in fact, alluded to the feasibility of extermination in the Khartoum area. *A. gambiae* could probably be exterminated easily in the Merowe Saras area by attacking its winter foci and moving further southward each year. It is doubtful, however, if there is enough malaria to justify the heavy expenditure on a large number of specially equipped motor vehicles and other transport which would be necessary.

MOSQUITOES IN GENERAL

South of Saras.—Between Merowe and Saras the only mosquitoes recorded are *A. gambiae*, *Culiseta longiareolata* Macq., *Aedes arabicus* Patton, *Culex tigripes* Grp, *C. univittatus* Theo. and *C. pipiens* ssp. *modestus* Forak. Only *A. gambiae* and *C. univittatus* are found commonly and then just in certain areas.

From Saras to Debeira—The above-mentioned four species are permanent inhabitants of this area. Larvae of *A pharoensis* were found at Abka in the Second Cataract in June, 1947, having probably spread from the north. *Aedes caspius* occurred at Wadi Halfa in the 1946 flood. A few larvae of *Aedes aegypti*, probably a chance visitor, were found at Wadi Halfa in April, 1944. *Culex theileri* has not been seen since it was found breeding in the Second Cataract in April, 1944. During the 1946 flood, when *C univittatus* was breeding in large numbers, some adults were noticed biting man indoors, an unusual occurrence in this species.

Faras—In the Faras basin the rate of seepage from the river has been reduced by partially filling an outlet canal. Breeding in the occasional brackish pools is controlled by pumping and applying larvicides, and in the canal by the continued use of *Gambusia*. *A gambiae* was last found on 6th December, 1942, *A pharoensis*, an immigrant from Egypt, in October, 1943, and *Culex theileri* in March, 1944.

Two species not previously recorded at Faras have been found, *Anopheles dthali* Patton and *Culex pusillus* Macq. Larvae of *A dthali* were found in December, 1945, and had probably spread south from Egypt. Larvae of *C pusillus*, probably also an immigrant from the north, were found in September, 1946, and May, 1947.

Records of *A multicolor* between 1942 and 1946 show that it has occasionally been found breeding in the basin in all months except July, August and November.

Culiseta longiareolata, *Aedes caspius*, *C univittatus* and *C p molestus* are occasionally found at Faras.

Neither *A dthali*, *A multicolor* nor *C pusillus* is known in the Nile valley south of Faras, which lies on the northern boundary of the Ethiopian region. The occurrence of these species is interesting in view of EDWARDS' (1941) remarks on their distribution.

SUMMARY

The reasons for undertaking the extermination of *A gambiae* are given. The work is of interest because the species was exterminated in an area which, though small, is a part of the Ethiopian region where *A gambiae* has occurred for many years, and because extermination proved to be very easy.

The probability of *A gambiae* having occurred in the area for many years is discussed.

The methods used, mainly dusting with paris green, are described.

Evidence of extermination is discussed, the absence of *A gambiae* during the exceptional inundation of 1946 afforded additional proof of extermination.

The effects of extermination are noted.

The riverain area upstream of Sarris, a possible source of reinfestation, is described, and other species of mosquitoes in the whole area are briefly discussed.

Present and proposed future work are described.

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NOTES ON ENDEMIC AND ACUTE MALARIA IN
CENTRAL AFRICAN NATIVES
by
J. SCHWETZ

Adults and children suffering from endemic malaria show wide quantitative and qualitative differences in their reaction to the disease. Quantitatively, in children the percentage of infection is much larger than in adults, qualitatively, in children in addition to *Plasmodium falciparum*, the dominating species, some *P. malariae* and even *P. vivax* are present, with gametocytes belonging to all three species. As a rule, in adults only the trophozoites of *P. falciparum* are found with, exceptionally, some very rare crescents.

Different districts exhibit marked differences in the rate and progress of the infection. In districts with a high malarial endemicity—hyperendemic districts—the peak of the infection is already reached at the age of 1 or 2 years, in districts with a low endemicity—hypoendemic districts—the most heavily infected children are much older, their age is 5 and even 7 or 8 years. The slowing down of the infective advance is accompanied by a slowing down of its retrogression. Moreover, the percentage of *P. malariae* infection in children is low, and *P. vivax* is almost completely absent in the hypoendemic districts.

Results of the examination of children soon after birth show

(a) No congenital malaria

(b) The first species to appear is *P. falciparum* (trophozoites and even crescents). Later comes *P. malariae* (schizonts and gametocytes), and lastly *P. vivax* (schizonts and gametocytes).

(c) The retrogression of the infection follows in reverse *P. vivax* disappearing first, followed later by *P. malariae* leaving in the adults only *P. falciparum*.

On treating a group of young children with quinine in a post natal health clinic for instance, there is a rapid, though temporary disappearance of *P. malariae* and *P. vivax* parasites (schizonts and gametocytes). *P. falciparum* parasites, and especially the crescents, are much more resistant and only succumb to much more intense and prolonged treatment with quinine.

I do not intend to discuss the controversial problem of malarial immunity a subject which does not come within the scope of this study. It may be concluded from the foregoing observations that the parasites of *P. malariae* and especially of *P. vivax* belong to a stage of recent infection, from which the organism gradually and spontaneously frees itself.

I now turn to the application of this deduction to the diagnosis of acute malaria. It is a well-known fact that cases of acute malaria arise from time to time even in districts where the disease is endemic—these must be due to a breakdown of the acquired immunity. This breakdown is more frequent in children, where immunity is most recent and therefore less stable than that of adults. But even among the latter acute attacks are to be found, due to lowered resistance from various causes such as fatigue, cold, removal from one district to another (superinfection, new strain, etc.).

The diagnosis of acute malaria in an adult native living in a malarial district is not always easy. Indeed, the malarial parasites are present both in healthy afebrile natives and in those suffering from fever. The intensity of infection and the large number of parasites usually accompanying a feverish attack are not absolute proof of it malarial aetiology for the parasites may sometimes be extremely numerous in healthy natives in certain hyperendemic districts, especially in children. Are these parasites survival of previous infection, or are they pathognomonic of a first infection? Can this be ascertained by microscopical examination or at least, can the use of the microscope bring a valid contribution towards such a decision?

On the other hand, malaria does not extend in a continuous belt over the whole of Central Africa. In some districts, or at least in some spots, no malaria at all is to be found, namely at heights over 1800 and especially 2,000 metres.

In these districts there is no more immunity among the natives than there is among the whites. In consequence, when the native inhabitants of these high districts come down into lower regions, where endemic malaria exists, they are not slow in contracting malaria and in reacting in a manner similar to that of Europeans with fever and the different complications, haemoglobinuria included. The industrial development of the Central African colonies has led to much migration amongst the natives, and this has been followed by severe outbursts of acute malaria.

This is especially true of Ruanda-Urundi, where foci of epidemic malaria have become established in recent years. The mean level of this country is high, but the land is very rough, the high inhabited plateaux being intersected by deep and marshy valleys. To prevent drought and consequent famine, the Government has drained and cultivated these marshy valleys, and this has been followed by epidemics of acute malaria among the non-immune natives of these highland regions.

Our recent investigations in Ruanda-Urundi have disclosed a highly complicated situation. On one side, on high plateaux of 2,000 metres with slightly lower valleys, we found no malaria except a few sporadic infections obviously contracted on trips to distant and lower districts, whilst, in lower districts of 1,400 to 1,500 metres altitude, we found a light endemic malarial infection. In regions situated at the high level limit of malaria, at about 1,700 to 1,800 metres, we found, besides a high endemic malaria, a certain number of recent cases of the acute disease, dating from the draining of the neighbouring marshes. Lastly, we found plateaux of 1,800 to 2,000 metres altitude, where cultivation of the neighbouring marshes at 1,500 to 1,650 metres had induced severe epidemics of acute malaria.

In certain of these districts the epidemics were not recent but dated back 2 or 3 years. The malaria was now subacute or subchronic, and fever was absent. The unravelling of this situation is not easy, especially the determination of the stage of the infection. Moreover, in some of these foci the whole population had been more or less treated with quinine and it was necessary to determine whether natives with no parasites had never been infected, or whether the absence of parasites was due to treatment with quinine. The parasite rates we found there were quite peculiar, bearing no resemblance either to epidemic or endemic malaria.

Two unusual phenomena had struck us and suggested the possibility of determining the "age" of a case of malaria by microscopical examination.

(1) In two neighbouring villages on the banks of Lake Edward (900 metres altitude), we found two entirely dissimilar malarial situations. In the first village the children were infected with *P. falciparum* + *P. malariae*, the adults harboured only some rare *P. falciparum* (trophozoites). In the second village

among the adults we found *P. falciparum*, some cases with *P. malariae* and even *P. vivax* (schizonts and gametocytes).

It was only later that we found the solution of this puzzle. The natives of the first village had always lived on the banks of the lake. Those of the second village had originally lived on the high plateaux dominating the western part of the lake, from which they had come down 2 years before in order to start a fishery. During the first year of their sojourn near the lake, these "immigrants" had suffered from fever and their death-rate had been inordinately high. At the time we visited them there was no longer a malaria epidemic, but merely the more or less immediate consequences of one, i.e., subchronic malaria, and this explains our findings of *P. malariae* and even *P. vivax*, among the adults.

(2) As regards the trophozoites of *P. falciparum* which form the bulk of malarial infection in children as in adults, in both endemic and epidemic malaria, I reproduce here a passage from one of our previous studies, written in 1939 and printed in 1941. After emphasizing the caution necessary in diagnosing fever of malarial origin in a district with endemic malaria, where the parasites may only be commensals, we made the following remarks:

"Nevertheless in most cases diagnosis may be made with great deal of probability especially among adults. If the pyretic patient shows no symptoms of any other disease, and if his blood at the same time contains large number of very small trophozoites, diagnosis of acute malaria may be made without any great risk of making mistakes. We insist upon the size of the trophozoites and upon the accompanying alterations of the red cells as a means of differential diagnosis between acute and chronic malaria, an adjunct rather than an absolute means, to be sure.

In this case there are numerous minute trophozoites, i.e., small thin rings, in normal red blood cells. Then, in chronic malaria we find more advanced trophozoites, with larger and thicker rings in cells having already suffered the specific alterations—a general coppery colour with certain number of 'Mearns' dots.

These two morphological varieties have struck numerous observers and to or even more sub-species of *P. falciparum* have been created accordingly. But in our opinion it is simply a question of age difference of the trophozoites. In acute malaria we encounter very young trophozoites which, owing to lack of time have been unable to produce alterations in the red blood cell. In chronic malaria we are faced with more advanced stages.

We may add that, even in children, a differential diagnosis may be made by a consideration of the morphological varieties of *P. falciparum*. But in certain hyperendemic districts of the Congo (in the Mayumbe for instance), the number of trophozoites (and this is equally valid for all malarial parasites) is very large in children whose state of health is nevertheless perfect. However the trophozoites encountered are large and thick, the globular alterations are so marked that the infected cell can be picked out at a glance, because of its deeper coppery

colour, even before the Stephens-Maurer dots and the trophozoites themselves are seen. On the contrary, in acute pyretic malaria the infected cells are not altered in the least and contain only minute trophozoites, very thin rings, but these are quite numerous, often several in one cell.

Although differential diagnosis between acute pyretic malaria and endemic apyretic malaria is fairly easy, complications appear in the case of subacute malaria, a sequel to epidemic malaria, the epidemic dating back several months or even 2 or 3 years. Patients are already sub-pyretic or even apyretic, although patently still suffering from their recent infection, not having yet acquired a sufficient immunity. It is this post-epidemic stage we encountered in different Ruanda-Urundi districts.

We must here distinguish between individual and collective examinations.

(a) *Individual Examination*—Presence or absence of numerous minute trophozoites in a child or an adult. The presence in an adult of *P. malariae* forms, and especially of *P. vivax* forms, is proof of a recent infection, of a sub-acute or subchronic stage.

(b) *Collective Examinations*—The results of the examination of a natural group of natives, such as a village, are clearer and easier to explain. In endemic malaria there is a sharp difference between children and adults, both quantitative and qualitative. This has already been discussed. No such difference is to be found between the different age groups in the case of epidemic or post-epidemic malaria. The number of infected individuals among adults is quite as large as, or even larger than, in children. The *P. malariae* and *P. vivax* parasites (schizonts and gametocytes) are found on the same scale in adults and children.

This picture is found only in untreated epidemics. Medication alters, in proportion to its intensity, the parasite picture. In a group of villages, for instance, when the epidemic had begun a few months before, and where the patients were found to be obviously ill, with or without fever, the population had been collectively treated with totaquina and atabrin, but in a haphazard and quite inadequate manner. The parasite picture we found among the patients was very curious, though easily understood: no, or very few, trophozoites, on the contrary, some rare or even fairly numerous *P. malariae* gametocytes, and especially *P. falciparum* (crescents, including young microgametocytes, circular in shape and red in colour).

SUMMARY

Although in general the malaria extant among Central African natives is endemic, in certain districts, namely, in the high plateaux bordering on the Graben (Kivu-Ituri and Ruanda-Urundi), epidemic malaria has been observed during recent years. The microscopical blood picture can be a useful aid to

the differential diagnosis of acute and endemic malaria. In the latter large and more or less thick trophozoites are encountered in cells with specific alterations in the case of acute malaria minute trophozoites are found which have not yet been able to cause alterations in the red cells

A notable distinction exists in endemic malaria as found in children and adults in children there is a remarkable proportion of infected red cells with *P. malariae* and *P. vivax* in adults the number of infected cells is very much less and only trophozoites of *P. falciparum* are encountered In epidemic malaria, pyretic or even apyretic, the number of infected adults is the same as that of children, and both groups show parasites of *P. malariae* and even of *P. vivax*

CORRESPONDENCE.

NOTES ON *SPIROCHAETA PERSICA* FROM PALESTINE AND *SPIROCHAETES* OF RELAPSING FEVER FROM THE WESTERN DESERT (TOBRUK AREA)

To the Editor, *TRANSACTIONS of the Royal Society of Tropical Medicine and Hygiene*

SIR,

Cases of relapsing fever occurred among soldiers in the Western Desert. The material for research was received from Colonel BOYD and Lieut-Colonel LITTLE, G H Q Middle East Command, for which we are greatly obliged.

(1) Thirteen strains of Tobruk spirochaetes were isolated from 12 patients and one tick. Guinea-pigs were highly susceptible to 11 of those strains but resistant to two.

After intraperitoneal injection of blood taken from cases during the first few weeks of the disease, the incubation period in guinea-pigs lay between 2 and 10 days. There was no uniformity of the course of the disease in guinea-pigs, even when several animals were inoculated with approximately equal amounts of the same infected blood, some had a heavy infection lasting about a fortnight which was not followed by any relapse, others had three and four relapses in the space of 2 or 3 weeks, and one animal exhibited the following infection as shown by daily thick drop examinations commencing the day after inoculation.

(— 3), (+ 2), (— 4), (+ 2), (— 2), (+ 3), (— 45), (+ 8), (— 1), (+ 3), (— 39)

(2) Contrasting with Palestine strains from which an animal can be considered to have recovered when its blood has remained negative for 1 month by thick drop examination, Tobruk strains can still be transmitted by the inoculation of heart blood 10 months after disappearance of the spirochaetes in thick drop examinations. Experiments with *S. persica* showed that animal blood ceased to be infective at the same time as the thick drop method of examination became negative, though with both strains it has been shown that the brain remains infective after the blood. The total period of infectivity of the brain with Tobruk spirochaetes has not yet been ascertained, but with Palestine strains the brain has been shown to be infected 20 months longer than the blood in rats, and more than a year longer in guinea-pigs. Moreover, with Tobruk strains it has been shown by animal inoculation that the blood is still infective for some time after parasites can no longer be demonstrated in it by thick drop examination, and in such cases the incubation period in the inoculated animal is increased to 1 or 2 months.

(3) Guineapigs which have recovered from Palestine strains remain immune to those strains. With Tobruk strains some animals develop specific immunity whilst others do not. In the latter cases a second infection with the same strain produced an infection equally as heavy as the first infection, in contrast to Palestine strains for which some cross immunity is developed. Thus an animal infected with a Palestine strain, if infected with a fresh strain, develops an attack less severe than the previous one.

(4) With Tobruk strains it was often noticed that the longer the incubation period in an animal, the more severely did it become infected. Some of the severely infected animals died with haemorrhages in the lungs, liver peritoneum and brain. A similar later termination often occurred in less severely infected animals if they were re infected with the same strain or with a fresh strain.

(5) *Ornithodoros tholozani* was demonstrated to be an experimental vector of Tobruk strains by feeding clean ticks on infected animals and subsequently reproducing the disease in healthy animals by feeding the same ticks on these.

(6) Lice fed on monkeys infected with Tobruk strains (to which guineapigs were susceptible), did not contain living spirochaetes demonstrable by inoculation of macerated lice at frequent intervals from 6 hours to 6 days after the last of their four feeds on infected monkeys.

Lice in which intra-coelomic injection of similar spirochaetes had been made were, however, infective by this method to guineapigs after a maximum interval of 48 hours.

Lice similarly infected with strains to which guineapigs were resistant became infected for a short period 18 hours after inoculation, but were negative 7 hours after and daily excluding the first day for 8 days.

I am, etc.,

Hebrew University

RYKA ASHBEL

LYMPHOSTATIC VERRUCOSIS

SIR,

I was very interested in Dr CLARK'S paper on lymphostatic verrucosis in Kenya (CLARK, 1948), and thought that photographs of a case seen at Mubera, Tanganyika Territory in 1948, might be of interest, particularly as the verrucose condition was marked and extended well above the ankle. Fig 1 and Fig 2 show different aspects of the same leg, the other not being so severely affected although it also showed scattered growths extending up to the knee.

The patient was a male African, of age about 35 to 40 years who attended for treatment for urinary bilharzia, and who refused to allow any interference with his leg condition as he said it caused him no inconvenience. In repeated examinations of his blood no microfilariae were found he had no signs of rashes

his Kahn reaction was negative, and he had no signs of leprosy nor suspicious organisms in nasal smears. Elephantiasis, yaws and leprosy were all common in the area, however. The surfaces of the warty growths were highly keratinized, and the condition of his feet resembled elephantiasis verrucosa (ASH and SPITZ, 1947).

I have often wondered since whether the condition might be a late manifestation of cercarial dermatitis in view of the fact that in this particular district, where lesser degrees of a verrucose condition of the feet were seen fairly frequently, bilharzia was rife. An examination of village school children indicated that 146 out of 207 were passing ova of *S. haematobium* in large numbers in their urine.



FIG 1



FIG 2

It is conceivable that cercariae might in some cases be held up in small lymphatics after penetrating the skin, and by the irritation of their continued presence cause proliferation of the epidermis at their points of entry, whilst if they were held up in the deeper lymphatics they would be liable to cause localized inflammatory reactions with fibrosis, eventually leading to an elephantoid condition.

I suggest that the possible association of verrucosis with bilharzia might be a line of investigation worth following up.

Alderley Ldge, Cheshire
13th December, 1948

Yours, etc,

T H WHITE

REFERENCES

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 ASH, J L & SPITZ, S (1947) *Pathology of Tropical Diseases* 266 London W B Saunders Co

VITAMIN B DEFICIENCY STATES

Sir,

With reference to the article by FINDLAY *et al* on Vitamin B Complex Deficiency States in West Africa, it is well known that the dietaries of the Gold Coast, Sierra Leone and Southern Nigeria differ markedly in the staple food stuffs, and as would be expected in the expression and symptomatology of deficiency disease. Again, only certain sections of the peoples show manifest evidence of these conditions the severity of which is primarily determined by economic circumstances, and it must be stressed that during the war years with the increase in the selling price of produce, malnutrition generally would be much less in evidence than in the inter war period, when economic conditions were so much worse. Indeed, this may account for some service personnel, posted to West Africa having failed to recognize nutritional and retrobulbar neuritis, formerly recorded there, as an organic disease—a belief that was dispelled by the return of prisoners of war (Far East).

Generally speaking, the Gold Coast is much less affected with frank nutritional disease than either Sierra Leone or Southern Nigeria. Though kwashiorkor (WILLIAMS) is more common there, neither the very extensive dietary and clinical survey of PURCELL nor of BEATRICE RUSSELL, have recorded any real incidents of nutritional retrobulbar neuritis there, in great contrast to these other two colonies.

Nutritional retrobulbar neuritis (followed by partial optic atrophy) can occur apparently independently on either beri-beri or ariboflavinosis. The signs of the latter will rapidly disappear with the return to a normal diet, including any keratitis which may have been present, but not so the central neuritic condition. In Southern Nigeria beri-beri is a practically unknown even unrecorded disease. Ariboflavinosis was excessively common among the manioc eaters, and associated with it, nutritional retrobulbar neuritis (in the active phase). Ariboflavinosis was, however not seen among the yam eaters, who only used manioc as a secondary staple foodstuff. The degree of ariboflavinosis varied considerably and in Nigeria was not as severe as among the rice-eating population of Sierra Leone. For example, frank keratitis was rarely seen in Nigeria but was exceedingly common among the Sierra Leone sailormen and krio boys, not infrequently leading to corneal ulceration. The incidence of nutritional retrobulbar neuritis in relation to ariboflavinosis (which in some schools was 100 per cent.) varied considerably. The highest ratio I found was 12 per cent. By contrast, among pregnant women a high percentage of whom (attending the Lagos ante natal clinic) showed ariboflavinosis, it was rare to demonstrate nutritional retrobulbar neuritis. These women viewed ariboflavinosis as almost part of their pregnancy and it rapidly disappeared following its termination.

H. H. SCOTT describing his central neuritis of Jamaica (an identical though more profound and acute condition to that in West Africa), showed

beyond all reasonable doubt that beri-beri, as a clinical condition, was not present in his cases. E J WRIGHT, in his description of the A and B avitaminosis of Sierra Leone, believed that condition as apart from beri-beri. The work of H S STANNUS, and many others, does not support beri-beri as a cause.

In the relatively few cases of "optic atrophy" referred to by FINDLAY and others, these authors do not state the duration of the condition, an important aspect with regard to response to any treatment. The findings of severely contracted fields is unusual and one wonders if there was not some added functional overlay.

Thiamin was found of little value in nutritional retrobulbar neuritis in prisoner of war (F E) camps. In his opening address at the Nutritional Conference, 1946, held by returned medical officers who had been either prisoners of war or civilian internees, SELWYN-CLARKE, the chairman, drew special attention not only of its failure to cure, but to prevent this condition. This statement was supported by many of those speakers. The writer, in Nigeria, was successful with autoclaved marmite which was as efficient as ordinary marmite, provided always, of course, the cases were seen early enough. The balance of opinion certainly does not favour thiamin as an agent of therapeutic value.

With respect to the somewhat conflicting views which from time to time have been expressed with regard to aetiology of this condition, in spite of the very real advances, by exclusion trials (made possible by advances in these diseases as a whole) clinical and dietetic surveys, etc., none the less it is a factor that the exact curative or preventative factor or factors is still not known. It is possible the cobalt-containing vitamin B₁₂ which, according to SPIES, possesses a powerful anti-neuritic effect, may provide an answer. But it is possible, also, it may not be a vitamin at all, but that "something else" present in marmite and yeast, and still unidentified.

"Hartearst," Gloucester Road,
Cheltenham
12th December, 1948

Yours, etc,
D FITZGERALD MOORE

----- INTESTINAL INFECTIONS OF PRIMATES

SIR,

Despite a natural reluctance to disagree with a former colleague, I must point out that Dr COCKBURN is in error in the penultimate paragraph of his article in your issue of November, 1948, on *Balantidium* infestation in primates. *Shigella* organisms do infect both apes and monkeys and in the former produce acute, blood-stained diarrhoea with extensive ulceration of the colon.

which is identical with human dysentery. In monkeys, however my own experience confirms previously published reports, *i.e.*, that although the colon and even the ileum may be ulcerated, diarrhoea is not a prominent feature the picture of a general septicæmia being produced, with rapid death. Only in the more chronic cases are the gut contents found to be fluid at necropsy. Most outbreaks have been seen in rather crowded cages where diarrhoea is difficult to observe in this type of case. Bloodstaining of the faeces does not seem to have been reported, however.

A further point is that *Sh. flexneri* has been found in primates several times and Col. BRIDGES and I reported another outbreak and reviewed the literature in the Bulletin of the Public Health Laboratory Service for January 1948, vol. 7 page 25. *Sh. schmitzi* has been reported once and recently I have seen an outbreak in apes and men due to this organism as yet unpublished. I am not aware that *Sh. sonnei* has ever been isolated from primates. It certainly never appears to have been found here as implied by Dr Cockburn.

I am, etc.

R. E. RUSSELL

Zoological Society of London.

5th January 1949

FOURTH INTERNATIONAL CONGRESSES OF TROPICAL
MEDICINE AND MALARIA

WASHINGTON, DC 14th May, 1948

Sir,

There is an old saying that it is a wise father who knows his own children that might be paraphrased to read "It is a wise society that knows its own members." On page 199 of the *TRANSACTIONS* for September, a footnote to Dr Barnes' paper refers to a luncheon party given by the U.S.A. Fellows of this Society. That should read *American* Fellows because the luncheon was sponsored by Canadians and Latin Americans, as well as United States citizens. This was a definite matter of policy—in invitation from the New World to the Old, and it was marked by the fact that the U.S. Fellows with the customary generosity that characterizes them, actually appointed myself a Canadian citizen—as Chairman. Dr BARNES gave the address partly in his capacity as the oldest Fellow in the New World. The original suggestion for the luncheon was made by Dr A. J. WALKER, of Tulane University (a graduate of McGill University, Canada), and it was received with the greatest enthusiasm by those Fellows of the American Society of Tropical Medicine, who were also Fellows of the Royal Society.

In justice, therefore, to the breadth of view of those who organized and attended this luncheon, I feel that this statement should be made. It was a special privilege to entertain our Fellow members from outside of America; it was particularly appropriate that the gesture should be a Pan-American one.

Yours etc.,

THOMAS W. CAMERON

(Local Secretary, Canada)

Institute of Parasitology,
Macdonald College, P. Q.
13th November, 1948

NOTE

This apparent discourtesy is greatly regretted. Unfortunately, the delegates from the Society did not realize that their hosts at the luncheon party included Canadian and Latin Americans, as well as United States citizens. The Society hopes that these two groups of their hosts will accept this apology with the same generosity as they showed at the function in question.

SPECIAL ANNOUNCEMENT

THE AMERICAN ACADEMY OF TROPICAL MEDICINE

(Office of the Secretary)

1420 TULANE AVENUE, NEW ORLEANS 15, LA., U.S.A.

The American Academy of Tropical Medicine has learned with great sorrow of the death of a distinguished honorary member Dr Charles Morley Wenyon, on 24th October 1948. Dr Wenyon was the leading medical protozoologist of his time who had the rare combination of scientific acumen and honesty and the warmth of human kindness. His scientific contributions were many and included both fundamental and applied investigations. He was a talented administrator. He received many distinctive honours, including the Theobald Smith Medal of our Academy in 1945. In his passing science has lost one of its great men and workers in tropical medicine, a broad minded and sympathetic colleague.

Resolved That the American Academy of Tropical Medicine at its Fifteenth Annual Meeting held at New Orleans on 7th December 1948, express its sorrow and extend its sympathy to Mrs. Wenyon and to Miss Mildred Wenyon and that the Secretary of the Academy write to Mrs. Wenyon and Miss Wenyon conveying this information and

Resolved That the Academy authorize Miss Mildred Wenyon to retain the Theobald Smith Medal awarded to Dr C. M. Wenyon in 1945 (1946), because of the devotion of Miss Wenyon to the cause of tropical medicine for so large a portion of her life

By direction

ERNEST CARROLL FOSTER

*Secretary**December 1948*

LIEUT COLONEL CLAYTON LANE

We regret to announce the death of Lieut.-Colonel CLAYTON LANE, M.D., F.R.S. (ret.). He served the Society as Honorary Secretary from 1925 to 1930 and as Vice-President from 1941 to 1943.

Owing to the prolonged illnesses of both Colonel Clayton Lane and Mrs. Lane ending in their deaths on 2nd January and 2nd December some of the requests for reprints of Colonel Lane's paper on Bancroftian filariasis have been misplaced.

Colonel Lane's daughter Mrs. Hodson, will be very glad to send copies to anyone who writes to her at Heathfield 1 Castlebar Park, Ealing London W 5

[The previous number of these Transactions, Vol 42, No 4,
was published on January 27th, 1949]

TRANSACTIONS
OF THE
ROYAL SOCIETY OF TROPICAL MEDICINE
AND HYGIENE

VOL 42. No 5 MARCH, 1949

ORDINARY MEETING

of the Society held at
Manson House, 26, Portland Place, London, W ,
on
Thursday, 20th January, 1949, at 7 30 p m

THE VICE-PRESIDENT,
Professor H E SHORTT, C I E , M D , Col I M S (retd)
in the Chair

PAPER

MALIGNANT MALNUTRITION (KWASHIORKOR)

BY

H C TROWELL, M D , F R C P , *
*Lecturer in Medicine, Mulago Medical School, Uganda, and Specialist Physician,
Uganda Medical Services*

In all parts of the world where poor people are poorly fed one or two dominant nutritional diseases occur, rickets among the children of the sunless temperate regions, and pellagra among the maize eaters in the Mediterranean type of climate Further, various infectious disorders appear to be common

* Thanks are due to Dr H LEHMANN, whose studies in haematology, but recently published, have considerably modified my views, and to Dr J GILLMAN whose histological material I was able to study and who thus demonstrated his idea of the life-flight Thanks are also due to Dr A R RAPER and to Dr J N P DAVIES for co-operation in pathological matters and to the Director of Medical Services, Uganda, for permission to publish this article

among these poorly nourished groups, respiratory catarrh among the rachitic children and gastro-intestinal infections among the pellagrins, so that it becomes difficult to decide whether the infection or the malnutrition is the primary condition. Tonight I wish to review briefly a malnutritional pattern seen in many parts of the tropics, and its relationship to tropical infections. For nearly 20 years I have been trying to consider this problem, many thousands of cases have been seen as in-patients or as out-patients, postmortem material among children now exceeds 100 cases of kwashiorkor. The disease has been studied personally chiefly in Uganda, to a less extent in Kenya and South Africa.

Definitions

Kwashiorkor (Cecily Williams, 1933) is one of the many African tribal names to describe malnutritional pattern commonly seen in African children, and occasionally in adults. Most writers have restricted this term to include only those who show the crasy-perement dermatosis and as such the term is used in this article. The syndrome is characterized by failure of growth, oedema, lowered plasma albumin, gastro-intestinal defect, anaemia, dermatosis and fatty liver. Dark races like Africans, show depigmented hair and skin. Cases have only commonly been described among Africans, few cases have been reported in whites in America, in Egyptians (HANARY 1947) and in Asians. Those who consider as I do that the crasy-perement dermatosis is pellagrous, may adopt the term of infantile pellagra, as being synonymous with kwashiorkor. Although I originally (TROWELL, 1937) proposed this term yet I do not now consider it suitable as it suggests that the condition is merely one of pellagra in infancy.

Malignant malnutrition in infancy (TROWELL and MUWALI 1945) is possibly wider term than kwashiorkor. It includes all those who have in severe degree several of the manifestations of kwashiorkor, but they usually do not have any crasy-perement dermatosis. A very large proportion of these patients in the tropics have also tropical parasitic disorders, but pneumonia and tuberculosis are also very frequent and very fatal, so that it is very difficult to decide how far malnutrition or infection, tropical or non-tropical, are contributing to the disorder. At any time if severely ill, crasy-perement dermatosis may appear and then most doctors would diagnose kwashiorkor. In my opinion no fundamental change has occurred, if so malignant malnutrition is almost synonymous with kwashiorkor.

A large number of African children and adults appear to show slight signs of this disorder. It does not appear suitable to refer to these people as suffering from malignant malnutrition or kwashiorkor and so it is proposed to call this chronic subnutrition and this is defined and described later.

RECENT LITERATURE.

It is impossible to review completely the literature of kwashiorkor, malignant malnutrition and chronic subnutrition. At the end of this communication bibliography as complete as possible of 125 articles will be given concerning these conditions. Almost all observers state that this disease or syndrome is not due essentially to tropical parasitic disease, that it is very common and very fatal and that it is as distinct clinical picture as pellagra, beri-beri, and scurvy.

It is now possible to indicate the geographical distribution of malignant malnutrition and Fig. 1 shows countries and areas which have reported cases in infancy. It will be noted that the syndrome is limited almost entirely to the tropics and as complete absence as far as can be ascertained, in the United States and the East Indies and the Pacific Islands must provoke thought, for there is much pellagra in the former as well as many negro children, and in all of these areas tropical disease is rampant. Its rarity in much of Asia has yet to be explained. Recently PARMON (1947) HANE (1937), RAMAN (1948) and

RAMALINGASWAMI, MENON and VENKATACHALAM (1948) have all described typical cases of kwashiorkor in India and it is now quite certain that this syndrome occurs in that country. Since the literature was reviewed by me in 1945 only one author, HUGHES (1946), has attempted to equate kwashiorkor with a vitamin deficiency disease.

WATERLOW (1948) studied especially those having an enlarged fatty liver and has made the most valuable contribution to our study of this aspect of malignant malnutrition. It is interesting that both he and ALTMANN (1948) arrived independently at the opinion that the old German paediatricians had described a very similar disease in *mehlnährschaden*, a disease attacking European babies in Europe, who had been fed excessively on starchy foods. WATERLOW, like ALTMANN and RUSSELL (1946) noted the great benefit conferred by milk, he also was the first to describe the abnormal histological appearance of the pancreas, and the voluntary muscles in this syndrome. DAVIES (1948) in Uganda, VEGHELYI (1948) in Central Europe, and CARVALHO (1945, 1946, 1947) in Brazil, have all independently described the pancreatic dysfunction which occurred in this syndrome and in nutritional oedema.

Very little work has been done on the late results of this syndrome. TROWELL (1948) has stated that many African adults show the later stages of chronic subnutrition and that this explains the anomalous results found by KEKWICK *et al* (1945) in their examination

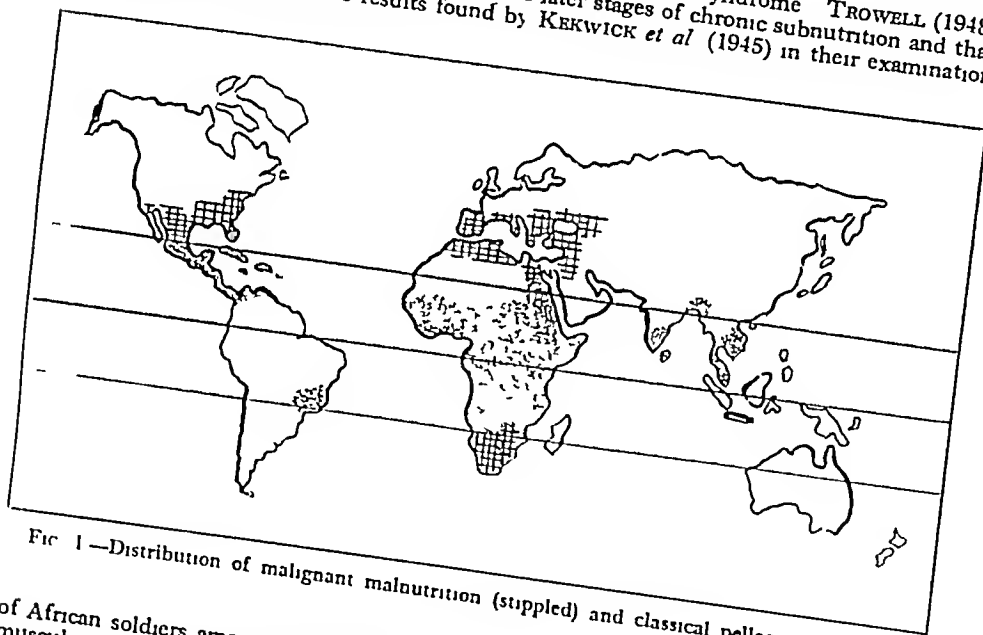


FIG 1—Distribution of malignant malnutrition (stippled) and classical pellagra (hatched)

of African soldiers among whom a fair proportion showed a low body weight, defective muscular power, a low haemoglobin, a low plasma albumin and a high plasma globulin, slightly soft hair, slightly cracked skin, and fine scarring of the liver, pancreas, kidney and so forth at many routine autopsies. Furthermore, KEKWICK found that although certain of these findings improved on liberal military diets, others did not improve much, that is they were refractory to treatment.

Finally, one may review the present state of our knowledge of the relationship of this syndrome to classical relapsing pellagra, considering especially the relationship of kwashiorkor (infantile pellagra) to classical pellagra in childhood. Fig 1 shows the different geographical distribution of the two conditions. Kwashiorkor is confined almost to the tropics, that is where vegetable staples predominate and tropical diseases are common, it

overflows into those sub-tropical areas, where maize is the dominant cereal and there may be marked seasonal change of climate. The latter are the areas of classical relapsing pellagra which is very rare in the really tropical regions of the world. With regard to the opinion of clinical workers, it can be stated that all, almost without exception, consider that kwashiorkor should be separated from classical pellagra in infancy although the changes in the skin in infantile pellagra are similar to those seen in classical adult pellagra (HARRIS 1941 and DODD 1941). ALTMAN (1945) reflects modern opinion when he states that the dermatosis is not an essential feature of the syndrome which can be distinguished from classical pellagra in its clinical picture, age-incidence, geographical distribution, response to treatment, mortality course and sequelae. GILLMAN and GILLMAN (1945) still incline to the view that the condition is one of pellagra in infancy: they have shown that the microscopical changes in the skin are identical with those found in classical adult pellagra: they have claimed that treatment by hog's stomach is specific, but this has not been confirmed by other investigators. Although various facets of the syndrome may improve when certain of the B vitamins are administered, there remains refractory core of malnutrition which yields only to good general diet and the treatment of all infections.

During the past 3 years my colleagues and I at the Mulago Hospital Medical School, Kampala, Uganda, have been actively prosecuting research into various aspects of this syndrome in children and in adults and much that I will now report is essentially due to their work and not mine. I wish to confine all my observations, except where otherwise stated, to the syndrome as seen in African infants in Uganda.

The Liver—This has been examined by biopsy in 181 cases, 40 of which were children under 10 years of age. The liver is always critically examined and usually sectioned at all routine postmortems, some 500 of which are performed annually. Some 50 of my cases, either children or adults, were examined by DAVIES (1948) and have been the subject of a special communication. In the acute attacks of malignant malnutrition, as seen in infancy whether cases have the crazy-pavement dermatosis or whether this is absent, a fatty liver is usually found, commencing as an accumulation of fat in the periphery of the lobule soon, however, in many cases the whole lobule is involved. It is probable that the liver is both larger and more fatty in malignant malnutrition.

TABLE I.

LIPOTROPIC ACTION IN MALIGNANT MALNUTRITION IN CHILDREN

Cases.	Daily supplements.	Lipotropic action.	Clinical results.
2	Nal	Slow improvement	3 very slowly improved
	$\frac{1}{2}$ lb. meat	Much	3 improved
3	$\frac{1}{2}$ lb. liver		3
3	1 pint milk		3
4	3 grammes hog stomach	Doubtful changes	2 2 died
2	choline	Very slight improvement	Doubtful response

From this it appears that all forms of animal protein are of benefit, of these if one may hazard any opinion on so small a series one would suggest that milk is the best.

in South Africa, where I was able to see Dr J GILLMAN's material, and in those described by WATERLOW (1948) in the West Indies. Lately, I have been considerably puzzled to find two cases of kwashiorkor in African infants with a typical dermatosis in whom no obvious excess of fat was detected in the biopsy specimen. The fatty liver has suggested that there might be a deficiency of the lipotropic substances which are essential for the efficient removal of fat from the liver cell, and of these substances choline, methionine, inositol and lipocic are those best known. GILLMAN and GILLMAN (1945) have made extensive claims for desiccated hog's stomach (ventriculin). It can be stated as a result of my 40 liver biopsies in children, that there is a very close correlation between the amount of fat in the liver and the prognosis in malignant malnutrition and in kwashiorkor in children, and further that fat disappears as the case improves. At first an attempt was made by liver biopsy, repeated at fortnightly intervals to assess the response of the liver to various forms of diet and various supplements, while at the same time clinical improvement was assessed. Only cases of acute malignant malnutrition in children were examined, in whom all infections (excluding helminthic disorders) were treated as soon as they were detected, and cases having a hypochromic anaemia received iron. Some cases (Group 1) were tested on the ordinary hospital diet ($\frac{3}{4}$ pint milk, 1 oz meat, and cooked plantains *ad lib*), others were given various supplements.

Considerable attention has been directed at biopsy and at autopsy to trying to understand not only the disappearance of fat from the liver but also the later phases of this malnutritional pattern. For details one can only refer to the results published by DAVIES (1948), but these seem to show that a fair proportion of thin stunted children and of thin stunted adults have all stages of transition from a fatty liver to frank portal cirrhosis of the Laennec type. One therefore gains a conception of chronic subnutrition, eventuating often in varying degrees of portal cirrhosis, and this conception is very different from that held by most workers in kwashiorkor, and it will be elaborated later in the conception of the deflected life-flight. Whether within this perverted development other abnormal reactions can be detected is not a subject on which enough data have been collected, but the following suggestions can be put forward. After 5 years of age a fair proportion of stunted children have a fine fibrosis extending from each portal tract and outlining the edge of the hepatic lobules, ascites and death from portal cirrhosis are uncommon in adolescence, but are very common in the twenties, especially in short, stunted persons. The relationship of this increasing amount of fibrosis to the incidence of jaundice during acute infections is not known, but infective jaundice from all infections and liver poisons is very rare in childhood, it becomes very common in adult life. Its mechanism and pathology are not clearly understood. Primary carcinoma of the liver is, as in all parts of Africa, extremely common and is considered to be another aspect of this cirrhosis problem (DAVIES, 1948), this

form of cancer is very rare in Europeans. One may refer in passing to the attempt made to examine the abnormal carbohydrate metabolism in adult cases of malignant malnutrition and the defective glycogenesis that occurs in the liver as studied by HOLMES and TROWELL (1948).

The Serum Proteins.

These have been estimated in many hundreds of cases of chronic sub-nutrition and malignant malnutrition in Mulago Hospital, and they have never fallen within the normal range in infancy. It is probable that this is the most constant abnormality detected in all cases and in all countries reporting kwashiorkor (infantile pellagra), malignant malnutrition in infancy whether tropical parasites are present or absent. The following generalisations appear possible.

(1) The serum albumin is always grossly reduced in malignant malnutrition in children and in adults, being usually below 2.0 grammes per 100 ml.

(2) The serum globulin is often normal in attacks of malignant malnutrition in childhood. It may, however, be temporarily raised, especially during the phase of recovery. In adolescence and adult life the plasma globulin tends to be raised, and these cases in adult life fall into two big groups, firstly the large majority in whom this change is reversible and whose clinical condition improves, and those with irreversible changes in the serum proteins and with a refractory clinical state and with fairly well established carcinomas detected in the liver biopsy.

(3) The great difficulty lies in evaluating the significance of those apparently normal African adults who show lower serum albumin and higher serum globulin than is considered to be normal in the European. The writer's personal experience of this is limited. It was found during survey of East African railway employees (TROWELL, 1945) and has been detected among African soldiers in both East Africa (LUXWICK, 1945) and West Africa (BLOURE, 1946) and these soldiers suffered no deficiency of calories or of animal protein in their diets and certainly had no nutritional oedema. The writer suggests that they were either examples of chronic subnutrition as discussed later, or were due to some unsuspected infection.

(4) Little is known concerning this rise of the serum globulin, which has not been resolved into its constituent fractions, but the thymol turbidity test suggests that gamma globulin is frequently very high.

ANAEMIA IN INFANTS.

When kwashiorkor is observed, dissociated from tropical parasitic disease, as in South Africa, or accompanying fatty liver disease in the West Indies, then anaemia is usually only moderate in degree and is usually normocytic. In Uganda one of our great problems has been that anaemia often becomes the principal feature of malignant malnutrition in children. In unravelling the aetiology of this anaemia great difficulty was experienced. We regard all African infants almost without exception, as having some malarial parasites (intense in the first few years of life) and varying loads of hookworms (at least from the second year of life from two to 60 to judge from worm counts after an anthelmintic or at autopsy on African infants). The anaemias which are ascribed to chronic malaria (normocytic orthochromic) and to hookworms (microcytic

hypochromic) are seldom seen, so that many have doubted whether these diseases cause severe anaemia and the tendency has been to call, very unwisely, all anaemia "nutritional"

The common anaemia of kwashiorkor in infancy in Uganda is a macrocytic orthochromic anaemia, normocytic anaemia may occur, so may hypochromia. This anaemia was thought to be a "deficiency" anaemia, hypochromia was due to iron-deficiency, the macrocytic element was less clearly understood, and it was considered to be allied to or identical with that operating in nutritional macrocytic anaemia. All attempts to elucidate the aetiology of this macrocytic anaemia failed, all sorts of liver injections were tried with poor, indefinite and erratic results. Folic acid and hog's stomach were clearly inactive, and the bone-marrow, at first considered to show some slight megaloblastic traits, was finally considered to be macro-normoblastic or normoblastic. The problem got stuck at this stage and it did not fall to me to find the master-key but to my colleague, Dr LEHMANN, who recently undertook research into anaemia in adults at Mulago Hospital. I do not wish to antedate his papers or constrain his opinions to fit my own, but quite independently I have been able to confirm his main contribution namely macrocytosis is not a sign of deficiency but an

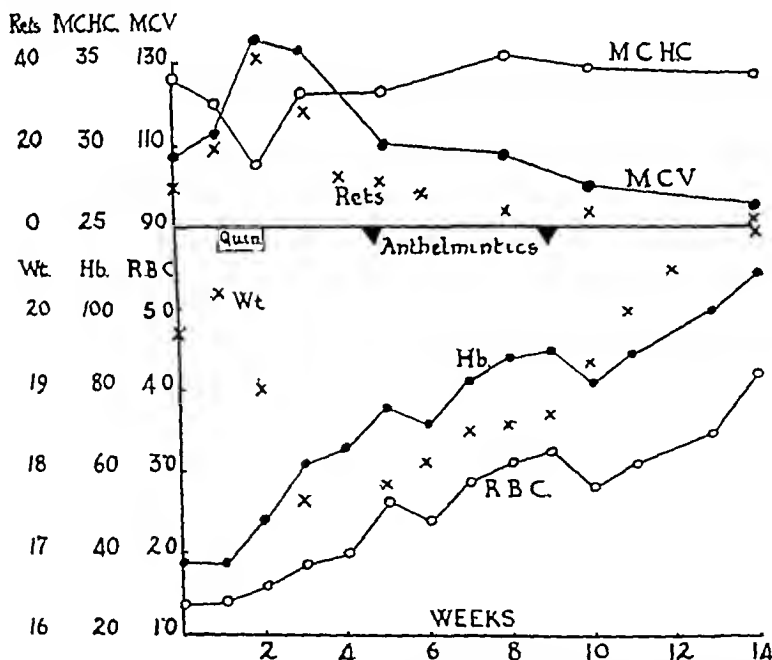


FIG 2 - MACROCYTIC ORTHOCHROMIC ANAEMIA. Severe MTM parasites, no treatment for first week, given quinine for second week only, with a response of macrocytic hypochromic reticulocytes, transient increase of macrocytosis and recovery impeded by two treatments of hookworms

anorexia, diarrhoea or vomiting, but milk can usually be taken. Drugs which increase anorexia and vomiting (quinine and sulphonamides) must be given sparingly. For the most feeble infants skimmed milk and blood transfusions will almost always rally the patient sufficiently to take ordinary milk, then gruels, and so forth. A good cereal such as brown bread is very helpful and is digested much better than vegetable staples (potatoes, plantains, etc.). All sources of animal protein should be high. Milk should be at least one pint, preferably two pints daily and if this is refused all other fluids are forbidden until the patient gives in. Meat, liver egg, fish and even groundnuts are all excellent.

(2) Examine repeatedly for all infections, especially the prevailing infections of the country and those known to have a high incidence in this syndrome. These patients bear with peculiar difficulty all infections which may appear to be very mild and negligible but no progress at all may be made unless these are detected and treated at once. Helminthic disorders are the only ones for which treatment may be deferred, but if there is reason to think that the infection is heavy and the haemoglobin is stationary treatment should be instituted. Many infections, such as those of pneumonia and malaria, are afebrile in this syndrome and may be accompanied by a leucopenia and are frequently missed. Probably the greatest advance in recent years in Uganda has been, firstly the employment of penicillin in bacterial infections, however trivial, rather than the sulphonamides which cause anorexia and vomiting and secondly giving daily paludrine, although quinine is still used for a few days if on admission the blood slide shows many parasites.

Finally bear in mind that there may be two "bottle-necks" in the race for regeneration, firstly protein, for it is difficult to give more than a few grammes of this each day this "bottleneck" should always be suspected if oedema is severe and stationary or the serum albumin is very low. To correct this we give dry powdered milk, like a medicinal powder several times a day. The second "bottleneck" may be iron in iron-deficiency anaemia but this is seldom present unless the hookworm load is heavy (over 20 in a young child). It must be stated with the greatest clearness that all vitamins are forbidden, so are unnatural foods like dehydrated liver, desiccated stomach, proteolysed meats, casein hydrolysates. These all distract time and money and attention. Everything must be directed to overcoming the false economy which would deprive wards of essential diets but would flood them with expensive vitamins and quack foods. All the time one must have in mind the parsimony of food votes, the conservatism of the kitchen, theft, maternal inertia and neglect by nurses.

DISCUSSION

In 1944 the conception of malignant malnutrition was set forth (TROWELL, 1944) as a syndrome, and not a disease, for it is probably not a clinical entity. It is a pattern the strands of the web are total calories protein.

vitamin B complex, the warp of the pattern has many different strands of an infective nature, helminths, pneumonia and so forth. The pattern produced is confused, but a certain drab monotony is seen in the clinical picture."

That still stands. When we examine by clinical, pathological and biochemical means children suffering from this syndrome cases do not seem to differ much whether they are seen in Uganda, where at least three-quarters have some tropical disease, and in Johannesburg, where almost no tropical disease exists, except that in the former anaemia is more severe, and tends to be macrocytic, the hair tends to be more red, the skin more depigmented, the liver less fatty and there is less dermatosis. The usual interpretation is that tropical infections complicate this syndrome, an alternative view is that many tropical and non-tropical infections can cause this syndrome, which may, however, arise as a pure nutritional defect. In fact, much sympathy must be confessed for those who state that this syndrome just does not exist, it is nothing but chronic malaria and hookworm disease and so forth in poorly fed persons, and that the older clinicians described all the signs of this syndrome under malaria, hookworm, ascariasis and so forth. That is undoubtedly correct. It is equally correct to state that various aspects of this nutritional pattern have been found, unassociated with tropical infections as in nutritional oedema, fatty liver disease, mehl'nährschaden and chronic pellagra and inanition. We do, however, need some concept big enough to cover all the facts: such that the child may be born under weight, that during the first few months of life, either due to inherited resistance or due to excellent breast feeding or due to less exposure to mosquitoes, malaria does not appear to be often a severe and prolonged strain, and this nutritional syndrome is almost never seen, and then about the time that breast feeding is given up comes the heaviest malarial parasitaemia, that this is seldom treated, that even if it is treated then even a trivial coryza may impede the regeneration of the blood, and that this underweight, anaemic child, with its fatty liver and depleted plasma and tissue proteins, is then handed on to various helminthic disorders, dysenteries and so forth, with episodes of respiratory infections, and that at no one particular moment can one say this case is just malarial disease and nothing else, or hookworm disease and nothing else or even nutritional disease and nothing else, likewise this child is sustained by a diet rich in carbohydrates and poor in protein. It is this pattern which is called chronic subnutrition and its acute phases, characterized by oedema and/or anaemia are called malignant malnutrition. It is not just the same as nutritional oedema, and Fig 4 represents some of the principal mechanisms which may operate.

When we examine at various stages developing African children and adolescents we are struck with the fact that all tissues affected most severely in malignant malnutrition continue to show slight signs of disturbance in a large proportion of adolescents and adults. Fatty liver to fine cirrhosis, atrophic pancreatic cells to chronic pancreatitis, crazy-pavement dermatosis to cracked

skin, anaemia continues, plasma protein pattern is disturbed and so is the endocrine balance. This opens up great possibilities which cannot be discussed adequately here, but, briefly speaking, there is evidence of a subtle deviation

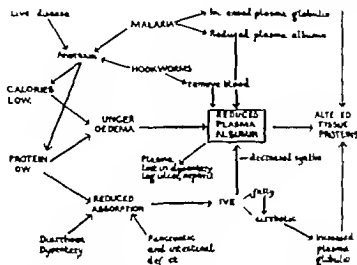


Fig. 4—Certain mechanisms operating to reduce plasma albumin in tropical undernutrition and malignant malnutrition

in normal development. Fig 5 represents the life flight, the trajectory of a normal child which should touch down at three score and ten. It should rise to a certain height, depending on the genetic plan and the momentum occasioned by good nutrition and upon infections being only a temporary affair from which full recovery is possible.

Chronic subnutrition is a deflected life-flight. Birth weight is often below normal, reflecting in many cases inherited malnutrition from the mother. Under the stimulus of excellent breast feeding the life flight soars during its first convalescent phase and avoids the premature crash of marasmic malnutrition as seen in bottle fed babies. Soon after this the engine stalls—breast feeding is prolonged, cow milk is minimal and mixed feeding is delayed and relies too heavily on one or two poor carbohydrates. At the same time, in the moist tropics, malaria from the first year and hookworms from the second year operate to disastrous results, and largely due to these two diseases the flight may fall into malignant malnutrition. How many seemingly normal adolescents and adults are short in stature, weak in limb, sluggish in mind, low in haemoglobin, with a peculiar plasma protein mosaic and a slightly abnormal liver, creatine, kidney and endocrine balance and so forth is not known. Nor is it known whether pneumonia and tuberculosis attacks more easily these subnormal

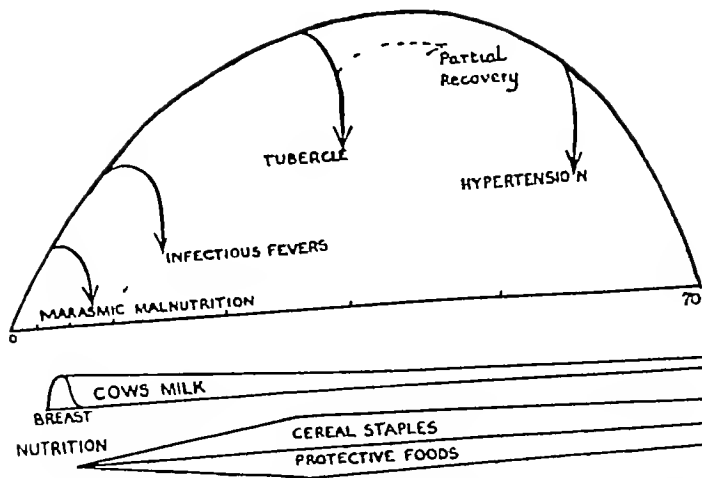


FIG. 5—NORMAL LIFE FLIGHT AND MARASMIC MALNUTRITION. Marasmic malnutrition is usually a syndrome due to failure to adapt to cow's milk and is often precipitated by pyrogenic infection.

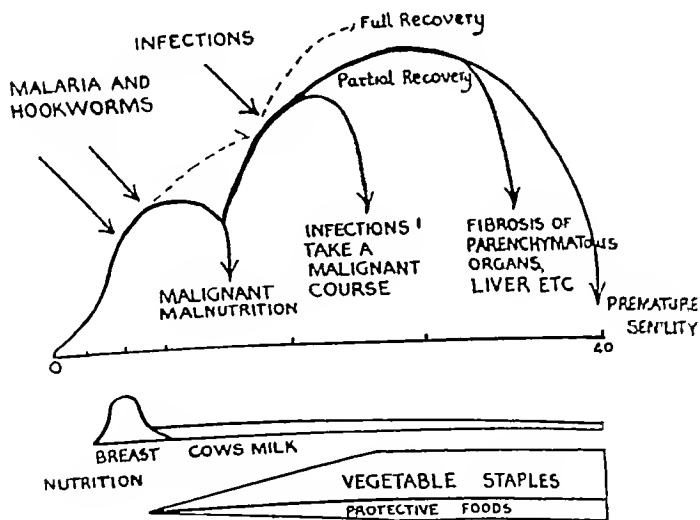


FIG. 6—LIFE FLIGHT IN TROPICAL SUBNUTRITION AND MALIGNANT MALNUTRITION. Malignant malnutrition is usually a syndrome due to inadequate food intake, especially cow's milk and suitable cereals after a period of weaning which has itself been delayed. It is often precipitated by tropical and by bacterial infections.

persons. Premature death and scilicet closes all too often this deflected life flight, the engine may peter out in premature cirrhosis, or ricochet into an infection, but even if these do not occur it may never rise to the height projected in the genetic plan.

Tropical subnutrition tends to become a deflected life flight.

SUMMARY

1 Malignant malnutrition, kwashiorkor and chronic subnutrition, are defined and the recent literature is reviewed. Almost all of the 85 different authors who have contributed to the literature of malignant malnutrition consider that it presents itself in childhood at least as a very serious and distinctive clinical picture with fairly consistent pathological changes. Most consider that shortage of first-class protein of animal origin is the most significant defect, together with excess of carbohydrate. There are many who suggest that the condition may occur as a pure malnutritional disease, unassociated with tropical parasitic disease or any bacterial infection.

2. Be that as it may there is much to suggest that in the tropics chronic parasitic and helminthic disorders most definitely cause, accompany and accentuate this disorder adding to it certain distinctive features such as severe anaemia and much depigmentation of black hair and of a dark skin.

3. In 40 cases of malignant malnutrition studied personally in Kampala, Uganda, in young African children, 19 showed malarial parasites in the blood slide, and in addition a further 12 showed malarial pigment in the liver biopsy thread. Hookworm ova were found in 25 cases, and other cases had other infections, affecting especially the respiratory and intestinal tracts.

4. The slow progress of the fatty liver to fine cirrhosis of the Laennec portal variety has been studied at 161 liver biopsies.

5. The lipotropic action of milk, meat and liver has been demonstrated and these are fundamental in any successful treatment.

6. The reduction of the serum albumin is probably the most constant feature of malignant malnutrition and chronic subnutrition in childhood.

7. When this disease occurs unassociated with tropical parasites, anaemia is but slight and is normocytic. Chronic blood destruction in malaria and by hook worms is apt to produce efforts at regeneration, with reticulocytosis and macrocytosis as features of the anaemia. Hookworm disease may also cause iron deficiency to occur and then the macrocytosis is "depressed" into normocytosis.

8. Treatment is usually very successful if all infections are treated vigorously even if they appear quite trivial and at the same time animal protein in an assimilable form (chiefly milk), good cereals, and fat are required.

9. The continued presence of chronic subnutrition causes subnormal development and abnormal signs at all ages. At first these deviations from normality can be repaired, later on this is not so.

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DISCUSSION

Professor Himsworth I think that I must be one of the few people in the room who have never seen a case of kwashiorkor, and I imagine that the reason I have been called on to speak is that I have been engaged in certain experiments concerned with one aspect of the syndrome. Without going into the question of the dietetic factors concerned in these experiments I may say that I, in common with many other workers, have found that a heavy prolonged fatty infiltration of the liver leads eventually to the development of a fine fibrosis throughout the organ which terminates in a condition indistinguishable from Laennec's cirrhosis. A similar type of fatty infiltration occurs in kwashiorkor, and the GILLMANS and DAVIES have shown that the same insidious development of fibrosis occurs in patients with this condition as occurs in the experimental animal. There thus seem to be good grounds for believing that both in man and in experimental animals the sequence which leads from heavy fatty infiltration to Laennec's cirrhosis occurs. In the experimental animal the requisite degree of fatty infiltration can easily be produced by diets deficient in lipotropic factors, including protein, and it is reasonable to suggest that a similar deficiency may play its part in causation of the same lesion in man. In connection with the fibrosis of the liver that develops in these conditions I should like to clear up one point. Laennec's cirrhosis is also known as portal cirrhosis, and the implication is that it arises from a proliferation of the fibrous tissue in the portal tracts. If one follows the evolution of experimental fatty fibrosis of the type mentioned one finds that the first fibrosis occurs round the portal veins. These are soon linked by prolongations of fibrous tissue and soon further links spread to the portal tracts. As soon as the portal tracts and the central veins are linked bile ducts grow down the fibrous strands and appear in the region of what were the central veins. At this stage it is quite impossible to say which vascular tracts were originally portal tracts and which were central veins. In the series of

sections of cases of kwashiorkor supplied to me by Dr GILLMAN of Johannesburg and of the series of sections from the livers of alcoholics with fatty infiltration, I have observed exactly the same sequence of events. It appears therefore, that we may have been deceiving ourselves in using the term portal cirrhosis and thinking that the condition primarily arose in the portal tracts. If after wider research, the results I have mentioned are further verified the position will be much simpler. It has always been difficult to understand how degeneration inside a lobule led to proliferation of fibrous tissue in the portal tracts. It is much easier to see how a degeneration in the centre of the lobule may lead to replacement fibrosis round the central veins and subsequent fibrosis. If this subsequent fibrosis is sooner or later invaded by new bile ducts the possibility of distinguishing such from portal tracts disappears.

Lastly I would just like to make a remark about the technique of liver puncture. Dr TROWELL uses the suction technique, by which a long needle is introduced into the liver and then suction applied by a syringe to aspirate the core of liver. I personally do not use this technique. I use the Vim Silverman needle, and in my opinion it is not only more certain in its results but it is very much safer.

Dr Waterlow. I have learnt a great deal from Dr TROWELL's paper. In the past there has been a tendency to concentrate on one or other feature of the kwashiorkor syndrome. I did that myself. In the laboratory in particular there is a danger of becoming one-sided. But as a result of what the workers in Kampala have been doing in the last few years, we have a much better basis for a balanced view of this complex disease.

If we accept the condition as primarily one of protein deficiency there are certain aspects which seem particularly worthy of study. The liver and the gut are two organs of primary importance since both are key points in the whole process of metabolism and nutrition. A disturbance in either or both, these key organs might well produce a multiple deficiency state, and so explain the diversity of the clinical picture so characteristic of malignant malnutrition.

It seems to me a reasonable supposition that the organs most severely affected in protein deficiency will be those that handle most protein. As far as I can find from the literature, the pancreas puts out in its secretion some five or six grammes of protein a day. The gastric juice may contain up to 10 grammes. We do not know the daily protein output in saliva and intestinal juice. Similarly the liver is turning out some 15 grammes a day of plasma protein alone. These amounts are large in relation to the daily protein intake of 60 to 70 grammes. Studies with isotopes have shown that the intestine and the liver have the highest protein turnover of all organs investigated. Thus, on the hypothesis put forward, liver and gut would be most vulnerable to the

DISCUSSION

effects of protein deficiency. An old observation is interesting in this connection in the dog fasted for 3 weeks the pancreas loses more weight than any other organ (62 per cent, compared with 40 per cent for the body as a whole). Atrophy of the pancreas has been produced experimentally in rats on a low protein diet, and IVY and his colleagues have shown that the enzyme content of the pancreatic juice can be altered by dietary means.

In the field, Dr TROWELL has made pioneer observations on changes in intestinal function. He has described alterations in bowel pattern, and defects of absorption in malignant malnutrition. DAVIES, in Kampala, has described fibrosis of the pancreas in kwashiorkor, and in the West Indies we found atrophy of the pancreatic acinar cells in infants with fatty liver. We know little about the functional state of the intestine in such conditions. Measurements of gastric acidity in the West Indies showed normal values. Diarrhoea and digestive symptoms are, however, common findings in many forms of deficiency disease, and may be severe. MCKENZIE, for instance, described diarrhoea as the most prominent feature of malnutrition in Tanganyika. Little is known about the cause of these disturbances, in spite of their importance to the economy of the body as a whole. For this reason I think that one of the most promising lines for the future is the study of the effects of malnutrition on the digestive system.

Sir George McRobert A condition clinically indistinguishable from African kwashiorkor is found in South India, where we have the same problems of malnutrition, combined with malaria, ancylostomiasis and amoebic infection.

My former colleagues, Professor GOVINDA REDDY, of Madras, and Dr GOPALAN, of the Nutrition Research Unit in Coonoor, are with us tonight. The former has brought sections demonstrating the infantile cirrhosis which forms such a feature of malignant malnutrition in South India, and you will agree, I think, that Dr GOPALAN's album of South Indian photographs shows a condition indistinguishable from that seen on Dr TROWELL's lantern slides.

I heartily agree with Dr TROWELL's recommendation to avoid vitamin preparations, to treat infections and to give an adequate diet. In many years of consulting practice my most frequent recommendation has been to cut out the many expensive Continental and American proprietary preparations usually prescribed by the attending practitioner, to spend six annas on iron and to use the money saved to purchase proper food.

With the opening up of Africa I suppose the poor patients there will also suffer from the pushing puffery of the manufacturing chemists which forms such a marked feature of medical practice in India.

Dr TROWELL's statement that progress may be hindered if infections which may appear to be mild and negligible are not treated, reminds us of

truth emphasized by Dr HAMILTON FAIRLEY in this room many years when he showed that quite a minor and undetected amoebic infection of the bowel may interfere with the specific treatment of a profound anaemia: that a few injections of emetine produced an immediate reticulocyte response with an acute rise of the erythrocyte and haemoglobin levels.

Dr Alan McKenzie There are three points arising out of Dr TROW'S most excellent paper that I would like to stress. Firstly that this condition is not merely an infantile disease from which complete recovery is possible; secondly that it is probably the manifestation of a general condition of malnutrition; and thirdly that protein, and animal protein especially, plays an important part in its cure and consequently, as I believe, in its prevention. I would also like to draw attention to Dr WATERLOW'S remarks on the relative power of the gut to absorb nutriment.

Some years ago I investigated in Tanganyika a not uncommon condition among adult labourers living on estates and receiving a ration that was generally less adequate and varied than that they were accustomed to in their own homes. The most prominent symptom was a severe and intractable diarrhoea and a skin condition similar to that shown in the photograph of the adult leg. At postmortem there was invariably a fatty liver superimposed on the almost universal African hepatic cirrhosis. There was rarely ulceration or marked signs of intestinal inflammation, but in nearly every case the wall of the gut was atrophic and lent colour to the clinical observation that these patients were incapable of absorbing a sufficiency of any diet offered. I came to the conclusion that either during their present illness or more likely during earlier life permanent damage had been done to liver and intestine and the condition was at this stage irreversible. These cases were then the final stage of a process that had started long ago and was related to a continued or intermittent state of sub-nutrition. Though no single dietary deficiency could be incriminated, it did seem that the lack of animal protein which is so general in African diets was a main cause.

I feel very strongly that we can explain a great deal of the stunted growth, poor physique and almost invariable hepatic cirrhosis by this continued lack of animal protein in the diet. For this reason I am anxious about the plans now afoot for the large scale export of meat from tropical Africa to this country. At present, over the greater area of Tanganyika the tsetse bars the way to the export of stock, if the new drug "anttrycide" makes large-scale ranching possible. The first care before we export meat to this country which though short, has at least more than its minimum needs, should be to make sure that the African has eaten enough and that he has not exported too much of that very animal on which he himself is most deficient.

DISCUSSION

Dr C C Chesterman I should like to comment on a region of Africa where this syndrome is comparatively rare I refer to a tribe of fisher folk along the Congo River below Stanleyville

The disease was found occasionally in motherless children or in twins who had been neglected owing to superstition

The customary diet for a weaned child was pounded fish and banana, and I should like to ask Dr TROWELL if he had found that a high protein fish menu was as effective in treatment as flesh diet

With no cattle, milk was only available from goats, and in a country where groundou is not uncommon the feeding of children on goat's milk was taboo lest it should produce horns They do not believe in homoeopathy but in homoeopathology—like producing like!

It was good to hear Dr TROWELL's remark about breast feeding A child astride its mother's hip, using the help-yourself-method until 2 years old, had the best prophylactic against malignant malnutrition

In view of the finding of the GILLMANS of fibrosis in the salivary glands in these cases, one would ask Dr TROWELL whether he has any evidence of this from Uganda

Dr Dean Smith Among the many points made by Dr TROWELL in his valuable paper, one of the most important is the aetiological complexity of kwashiorkor Opinions have swung between malnutrition and helminthiasis as sole causes Dr TROWELL has shown the importance of hookworm in the presence of an apparently adequate food supply, WATERLOW's cases of the same condition had no evidence of helminthic or protozoal infestation, we have studied cases in the Gambia in which the protein intake, both total and in amino-acid pattern, so far as we can ascertain it, is apparently just adequate to support normal individuals, yet the load of infection and infestation renders it insufficient

In describing kwashiorkor as a nutritional disease it is important to interpret the term "nutritional" as implying a cellular metabolic defect, whether this arises from insufficient intake, failure of digestion, absorption or utilization or from increased requirement due to such stress factors as worms or malaria

Brigadier J Bennet Three years ago I found myself drawing analogies between cases seen in a prisoner of war camp in Formosa and the clinical picture drawn by Dr TROWELL in his earlier work Malnutrition was associated with a diet consisting of unpolished rice and coarse, unpalatable vegetables with scanty and infrequent supplements of meat and soya bean products Its caloric value was estimated to be approximately 1,800 calories although it was higher for considerable periods An impression was formed that a reasonable state

of health might have been maintained but for the presence of certain factors conditioning deficiency disease, mainly the compulsion to hard work, the prevalence of diarrhoea and, to a lesser extent, malaria. The main feature of the cases shown in common with those Dr TROWELL has described was oedema, but in a few in whom diarrhoea was severe and recurring cracked skin with mosaic pattern on the anterior aspect of the legs was seen.

Dr L. E. Napier I must first congratulate Dr TROWELL on his excellent description of a disease syndrome that I probably overlooked, as such, for 25 years in India. I can now recall many cases that would fit his description, but the nearest we ever got to a diagnosis was "infantile pellagra." Although there is a marked similarity in the pathology and epidemiology there is little in the clinical picture to associate kwashiorkor with "our" infantile biliary cirrhosis.

I should, however like to ask Dr TROWELL if he could explain one statement that he made and which I fail to understand, namely "macrocytosis is not a sign of deficiency but an aspect of blood regeneration" the word "macrocytosis" is unqualified. I cannot agree to this as a general statement.

Ever since the reticulocyte was recognized and named, it has been fully appreciated that it is a larger and paler cell than the mature red cell. Allowance has always to be made for this fact in judging whether an anaemia is macrocytic, normocytic or microcytic, and for this reason it is always preferable to make a diagnosis before treatment has been started.

The larger size of the reticulocyte is very well brought out in a paper by Dr P. C. SEN GUPTA and myself—*Indian J. Med. Res.* 31: 75—although this was not the purpose of the investigation.

Colonel E. P. M. N. Early Perhaps a few words on the ward management of cases of malnutrition and anaemia would interest the Society. In 1946 I was in command of an Indian Base General Hospital (II) for Indian troops in Bareilly U.P. India. In this hospital we had 75 beds devoted entirely to these cases.

I cannot go into clinical details as this aspect of the cases was in the capable hands of my Officer-in-Charge, Medical Division of the hospital, who is not with us tonight.

A base general hospital is usually the last port of call of war casualties, where they are treated to a finish. We had patients in this ward of all classes and creeds in India and most were very ill on arrival.

Total R.B.C. counts were often between 1 and 2 million per c. mm. hb. 20 to 30 per cent. and weight down to 50 to 70 lb. Often there was gross oedema of face and ankles and little desire to survive or to eat or drink. In such cases treatment was usually started in a side ward with intravenous saline, glucose

DISCUSSION

and plasma. The greatest struggle was to get the patient to eat and drink. I found it nearly always possible to get a Briton to take nourishment even when he does not feel like it. The Indian won't. "He is not hungry," "Not thirsty," and he just won't. We found that half the battle was over once the patient started to take food by mouth. When the immediate risk to life had been dealt with by rectal and parenteral therapy, and he had been coaxed or persuaded to take something by mouth, he was moved to the main ward and placed next to our plump and well fed, more successful patients.

The atmosphere in this ward was an exceedingly happy one as all the patients could watch their own progress in appetite, weight and general well-being.

I must say a word regarding two points that cropped up in treatment. A proportion of cases reached a stage when the percentage of hb and total RBC remained stationary below the normal level, in spite of parenteral crude liver extract, iron therapy and good mixed feeding. A preparation of proteolysed liver given by mouth in powder form (one to two teaspoons t.i.d.) seemed to work like a charm producing a reticulocyte shower and rapid improvement in the whole picture.

We also found in the early stages of parenteral therapy a small blood transfusion of about 500 ml produced an improvement out of all proportion to its size.

Dr K P Hare I think, as Dr NAPIER has pointed out, there is a danger in stressing the nutritional factor too much in this syndrome. In fact, it has been said by some speakers this evening that the basic factor in kwashiorkor is protein deficiency. I feel doubtful about that. In the cases I have described arising in Assam, I think I showed clearly that we were only too much acquainted with lack of proteins in Assam. Nutritional oedema was always with us, and the condition of intractable diarrhoea was by no means infrequent, it was seen in the evacuees from Burma on the Manipur road but those people did not show any of the symptoms of kwashiorkor. There is a danger in stressing the nutritional aspect, and particularly the animal protein intake, when considering this disease in that you may include and classify as kwashiorkor a large number of conditions which have nothing to do with it. I think that, though kwashiorkor may not be a disease, it is a very definite syndrome, and when considering cases they must be shown to include all or most of the leading symptoms. I feel this new teaching that crazy pavement dermatosis is not necessarily a symptom of kwashiorkor is perhaps a mistake.

Dr Trowell (in reply) I must thank those who have spoken, and I will endeavour to answer some of the points raised. Professor HIMSWORTH raised the question of whether portal cirrhosis is not a misnomer because in his experi-

ments in animals the fibrosis starts rather more around the central veins of the hepatic lobules. In one of our liver biopsy specimens from a child of 3 years, shown here tonight, Professor HIASWORTH points out that there is a fair amount of fibrosis around the central vein as well as around the portal tracts. On the other hand, I would state that our experience at Mulago inclines us to believe that fibrosis predominates around the portal tracts—soon, however it is evident around the central vein and is infiltrating between the columns of liver cells. Our great difficulty is to follow over a long period of time the sequence of events in untreated cases in human beings. We are under an obligation to treat all cases as soon as they are observed and it is difficult to do serial liver biopsies on untreated cases over a space of several years and thus to watch the progress of cirrhosis. Liver puncture biopsy has come in for a good deal of criticism, and before I reply to this point I would like to thank those patients whose courage allowed them to consent. It is performed under general anaesthesia, pentothal in adults and ethyl chloride in children, so there is no question of undue pain. It carries a very definite risk and can only be justified in rather unusual circumstances to obtain knowledge which cannot be found in any other way. In this syndrome among children the mortality has been set as high as 50 per cent. Elsewhere I will publish the technique employed in 200 cases and the difficulties and risks encountered. Nowadays the mortality is under 10 per cent. and very substantial gains in knowledge have been made by liver biopsy.

Dr WATERLOW pointed out how hypoproteinaemia would render difficult the formation of many enzymes, including those in the pancreatic and intestinal secretions, so that there might then be difficulty in breaking down and absorbing food, thus accounting for the undigested food in the stools and for the absence of zymogen granules in the atrophic pancreas. I have always considered this to be a very suggestive point, and it may explain why advanced cases fair so poorly on an indifferent native diet, containing rather large pellets of potatoes and so forth in the intestine but benefit so much from milk. The latter and possibly also finely milled cereals can be tackled by even small amounts of pancreatic and intestinal enzymes, which are unable to tackle a large piece of potato or meat. Certainly if one of the undigested pellets of carbohydrate is obtained from the stool and tested with odine digestion can be seen to have been fairly good on the surface of pellet, but it has not started in the interior of the mass. Thanks to the help of my director Dr AUSTIN who, like all my directors, has been most helpful, we hope in Uganda to investigate in the near future the production of intestinal and pancreatic enzymes, and I am glad to hear that Dr WATERLOW will be looking into other aspects of this problem on the West Coast.

I am pleased to see the photographs shown by Sir GEORGE McROMERY on Kwashiorkor in India—children and to hear the remarks of Dr NATHAN

both stressed that this syndrome is common in India but unrecognized until recently

I was very impressed by what Dr DEAN SMITH had to say about his experiences in Assam and in West Africa. In the latter country protein intake in some of the diets appeared fairly adequate and the question arises whether this disease is nutritional in origin and whether it is a single clinical entity or pattern. In other words, is this condition due to a dietetic deficiency and should kwashiorkor cases having the pellagrous dermatosis be separated from those having no dermatosis? My own feeling is that we must start with ascertainable defects in the cells and fluids of the body. There certainly is a hypoalbuminaemia, and this would suggest that there are changes within the protoplasm of the cells since this is inequilibrium with the plasma proteins. There is malnutrition cellular protoplasm in terms of protein, there is a nutritional defect at the cytological level, this is not synonymous with a diet poor in protein. The position is parallel to that found in iron-deficiency anaemia, this may be dietetic in origin, although this is seldom true in men, more often there are difficulties in absorption in its transport and storage, or there are abnormal losses from the body by haemorrhages. In all these conditions the plasma iron is low, there is a defective and retarded and abnormal type of haematopoiesis, and almost all cases will improve when given iron by the mouth, all will improve if a suitable form is administered intravenously. In all cases there is a deficiency of iron at the psychological level and the end results may be very similar. Dr DEAN SMITH would also ask if cases of kwashiorkor having the pellagrous dermatosis cannot be separated from those showing no signs of vitamin deficiency, my own view is that advanced cases are running short of many vitamins depending largely on the basic diets and the prevailing infections, but that any fundamental division of this syndrome along these lines is misleading and misses the fundamental basic unities of pathology, prognosis and treatment.

It was interesting to hear from Dr CHESTERMAN that kwashiorkor is uncommon along the banks of the Congo river, where there is, presumably, much malaria and hookworm disease, but also much fish is taken. This supports the contention that if protein intake is high, almost indeed excessive, one can contend well with a heavy strain from parasites, which are themselves suppressed by the rising immunity of the host. Those children who appear to be lightly infected and may not develop kwashiorkor may escape it by reason of excessively good nutrition decreasing the parasite load. There is much to show that kwashiorkor is rare in the really primitive parts of Africa where, however, there is much tropical disease, but becomes common in advanced centres where food is bought, the soil eroded, family life has been disrupted and that this syndrome reflects the stresses of a changing community.

Dr NAPIER asked about the relationship of macrocytosis in the anaemia

to deficiency and I am only sorry that Dr LEHMANN is not here tonight to answer that. For my own part, I cannot attempt to answer it, but now that his paper is published some comment on his valuable contribution is permissible. The reticulocyte is usually larger than the normal cell but haemolytic anaemia, with its accompanying reticulocytosis, is seldom macrocytic. WESTROB classifies these anaemias as normocytic. It is also certain that several papers testify to the fact that hookworm anaemia in America and in India is usually quite definitely microcytic and hypochromic, and that when hypochromic anaemia occurs in a European (from any cause) it does not become macrocytic during the recovery stage. We therefore have to ask why a different state of affairs occurs in Africa and, in my opinion, much of the work on nutritional macrocytic anaemia in Africa and Asia will have to be re-assessed. In my opinion under prolonged strain from blood destruction in malaria and hookworm the bone marrow develops what is being called a hyperplastic reaction which is often macro-normoblastic and produces reticulocytes and red cells which are both larger than those produced by a normal marrow. This macrocytosis does not apparently reflect deficiency of any of those substances which aid recovery in pernicious anaemia but reflects a chronic strain set possibly against the background of malignant malnutrition: once these diseases by themselves do not in Europeans or Asians produce exactly the same kind of anaemia.

COMMUNICATIONS.

SOME OBSERVATIONS ON THE ACTION OF QUININE, ATEBRIN, AND PLASMOQUINE ON *PLASMODIUM VIVAX*

BY

M J MACKERRAS

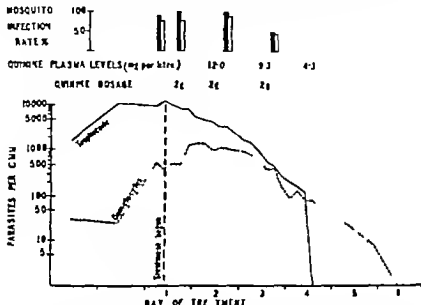
AND

Q N ERCOLE

Quinine and atebtrin have long been recognized as efficient schizonticides in all forms of human malaria, and it has been customary to regard them as having a gametocidal action in vivax and to a less extent in quartan malaria. All workers agree that neither possesses any action on mature *Plasmodium falciparum* gametocytes. Plasmoquine, on the other hand, is usually regarded as a poor schizonticide and a good gametocide. There is ample evidence of its destructive action on mature gametocytes of *P. falciparum*. This we were able to confirm, but we failed to demonstrate any direct gametocidal action in quinine or atebtrin. Plasmoquine was found to have some action upon all erythrocytic stages.

FIELD (1938) reviewed the various hypotheses which had been brought forward to account for the action of quinine. Some authors stated that it acted only on the merozoites free in the plasma, while others, impressed by its failure to kill parasites *in vitro*, postulated that its action was indirect, *i.e.*, in stimulating the defence mechanism of the host. JAMES (1934), however, had already described the changes in appearance and staining that occurred in parasites within the red cells when patients with vivax malaria were given atebtrin, but

day of quinine therapy although by then the count was usually too low to render such attempts practicable. It was not unusual for the gametocyte count to rise during the first 24 hours of quinine therapy as it did in TOU (Graph 1) but from then on the count invariably decreased. It seems likely that those gametocytes, which appeared in the circulation early in treatment, had already completed their development before the drug could affect them.



GRAPH 1. Parasite densities and mosquito infection rates in TOU treated with Q.A.P.

Histograms above represent infection rates in mosquitoes fed 1 times indicated. Solid columns indicate gut infection rates and stippled columns sporozoite rates.

On the other hand, we consider that quinine inhibited the growth of young gametocytes in the same way that it affected the trophozoites. Those merozoites destined to develop into sexual forms never reached the stage of being recognizable as gametocytes.

(*) GRU Treatment was begun when the trophozoites were little more than half grown, and was limited to quinine sulphate gramine given in mixture in three divided doses at 2-hourly intervals.

Before quinine was given, trophozoites numbered 12,000 per mm. they were mainly large amoeboids (Fig 2 f) with an occasional schizont. Gametocytes numbered ~,000 per cmm and exflagellation was observed.

Four hours after the first dose many trophozoites were affected. Amoeboid activity

Q.A.P. = Standard course of treatment, namely quinine gramine 2.0 daily for 3 days ateban in decreasing doses from gramine 0.6 to gramine 0.2 daily for 3 days quinine gramine 1.0 and plasmoquine gramine 0.03 daily for 3 days.

was lessened, and contraction of the cytoplasm, distortion and even separation of the nucleus was observed in the stained film (Fig 2 g). Some new rings had come into the circulation since the pre-drug film had been taken, indicating that mature schizonts might sporulate, and merozoites invade red cells in the presence of quinine. These young rings, however, were already affected by the quinine.

At 6 hours the majority of the amoeboids were immobile, and, in the stained film, showed considerable changes, e.g., premature division of the nucleus, loss of even contour, or fragmentation of the cytoplasm. These changes were more pronounced at 8 hours (Fig 2 h). At 12 hours no amoeboid activity was observed, though the pigment still showed oscillatory movement. At 20 hours the trophozoites were contracted, sometimes with extrusion of coarse lumps, and abnormal nuclear divisions had occurred, the pigment aggregated into coarse lumps, and irregular vague streaks of chromatin were present. The young pale, ragged cytoplasm and irregular narrow bands or small dots of rings, which were first observed at the fourth hour after drug, had not grown appreciably and appeared as irregular wisps of cytoplasm, with dark, narrow bands or small dots of chromatin. They were similar to the distorted rings and young amoeboids seen in films from TOU (Fig 2 b).

The trophozoite count during the first 6 hours increased slightly, due to the presence of new rings which were forming at the beginning of therapy. It then remained more or less stationary until the 20th hour, but, between the 20th and 24th hour, a sudden drop to 5,000 per c mm occurred. At the same time the patient experienced fever and chills similar to those accompanying a schizogony.

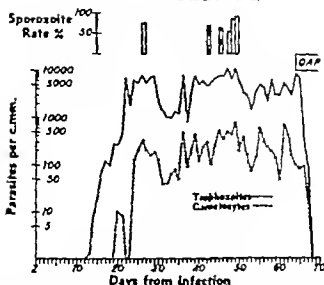
At 32 hours one mature schizont was found in a thin film. It had three complete merozoites, two of which possessed very little chromatin, but the third appeared quite healthy, though larger than normal, a residual mass of cytoplasm, chromatin and pigment was also present (Fig 2 j). One rosette with four merozoites was found in a thick film, but no new rings were seen in films taken at 32 or 45 hours after the first dose of quinine.

Forty-five hours after the first dose and 41 hours after the last dose of quinine, the count had dropped to 190 trophozoites, the majority of which were degenerating, and 120 gametocytes per c mm. Full therapy with paludrine and plasmoquine was then begun. Gametocytes decreased gradually during the first 24 hours, and then declined rapidly. Exflagellation was observed at each examination from 6 to 26 hours after quinine, but was not sought for later than this period.

(b) *Suppressive Dosage*

Quinine, administered in daily doses of gramme 0.33 or gramme 0.66 to volunteers exposed to infections with *P. vivax* (sporozoite-induced), usually failed to suppress the infection, and trophozoites and gametocytes appeared in considerable numbers in the blood stream. This occurred in three out of three volunteers on gramme 0.33 daily, and in three out of four on gramme 0.66 daily (FAIRLEY, 1945).

Batches of mosquitoes were fed, and infections obtained at each trial, on two of the volunteers taking gramme 0.33, and on one taking gramme 0.66 daily. The latter patient, SMI, had demonstrable parasites, but remained ambulatory. Full treatment was not begun until the 65th day after infection. During this period of 65 days trophozoites were present continuously from the 3th day, and gametocytes from the 20th day. On 22 days gametocyte counts in excess of 200 per c mm were recorded, the maximum count being 730 per c mm. Mosquitoes were fed on this patient six times, and infections obtained on each occasion (Graph 2).



GRAPH 2. Parasite densities and mosquito infection rates in SMI on quinine granules 0.03 daily.

It is apparent from the graph that small doses of quinine merely diminished the rate of multiplication of the parasites, presumably by eliminating those individuals most sensitive to its action. The epidemiological significance of these observations on the infectivity of the gametocytes is obvious.

ATEBRIN.

(a) Therapeutic Dosage.

PETER (1932) was one of the first workers to record observations on the action of atebirin on *P. vivax*. He considered that it first destroyed the rings, then the schizonts and finally the gametocytes. JAMES (1934) described and figured degenerative changes in the cytoplasm and nucleus, together with abnormal clumping of the pigment, and its expulsion from the cytoplasm.

We observed the effect of atebirin on several patients treated with atebirin dihydrochloride granules 1.0 on the first day followed by granules 0.4 daily for 6 days. Details of two of them are given below.

- (1) S.L.A. This patient had tertian fever and treatment began while schizonts were occurring. Immediately before therapy the blood contained rings (Fig. 3 a), some half-grown amoeboids (Fig. 3 b), one full-grown trophozoites and few schizonts. No gametocytes were seen.

Two hours after the first dose of atebirin granules 0.5 the half-grown amoeboids were still seen, but showed abnormal clumping of the pigment, and occasionally fragmentation of the cytoplasm (Fig. 3 f). The premature coalescence of the fine pigment granules was well seen in stained films. Some young rings appeared normal, but in many the cytoplasm was pale and indistinct, the nuclei were still round and compact, and stained very darkly (Fig. 3 b). Large trophozoites and schizonts were scanty, but all observed showed clumping of the pigment. After 4 hours the above changes were more marked, and in some of the larger parasites the pigment was completely extruded from the cytoplasm (Fig. 3 i). No amoeboid movement was detected in wet preparation.

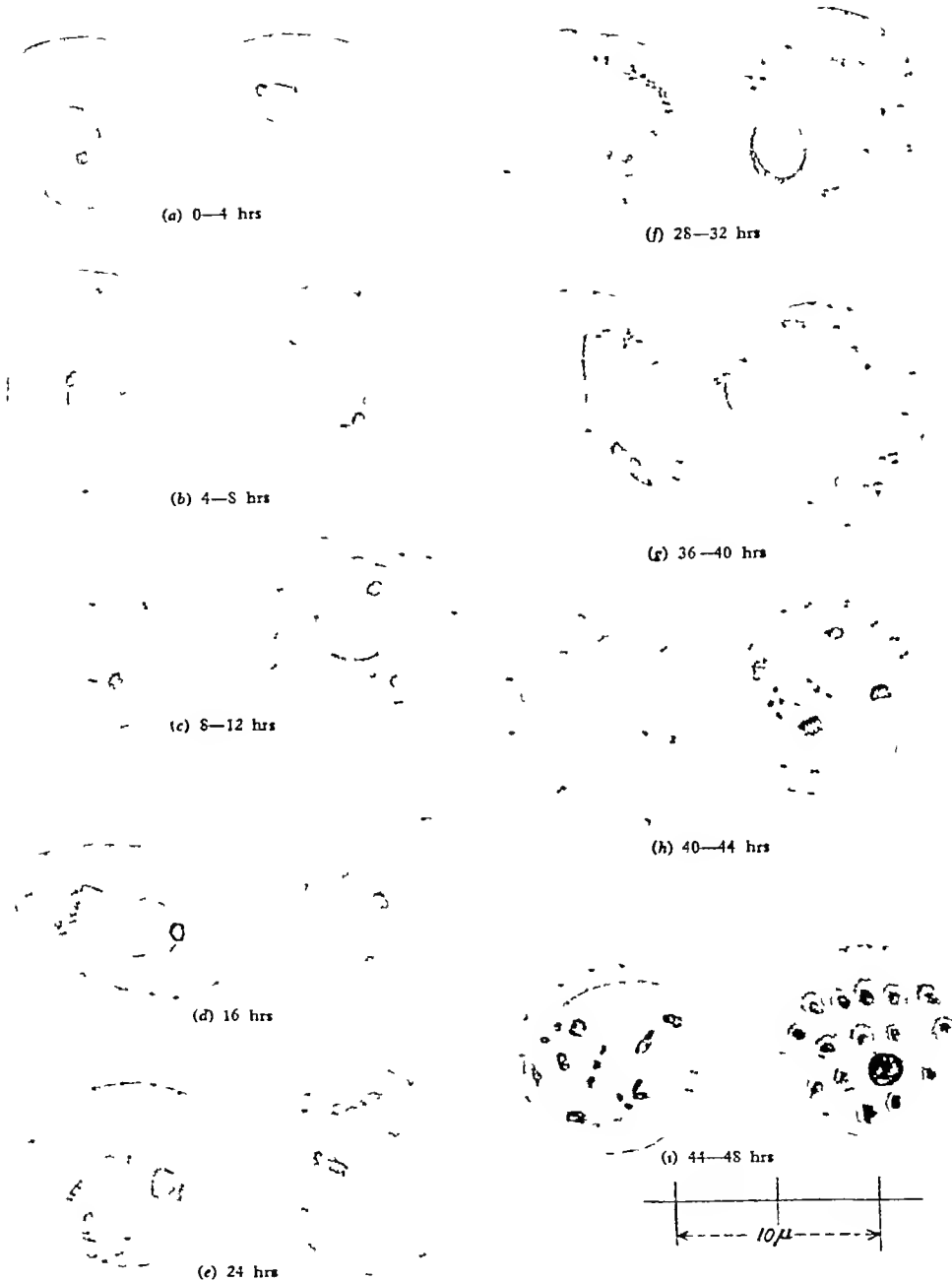


FIG 1 *P. trax*—normal growth of trophozoites

- | | | |
|--|-------------------------------------|--|
| (a) Young rings | (d) Amoeboids 16 hours old | (g) Preschizonts 36—40 hours old |
| (b) Rings and young amoeboids 4—8 hours old. | (e) Amoeboids 24 hours old | (h) Early schizonts 40—44 hours old |
| (c) Amoeboids 8—12 hours old | (f) Large amoeboids 28—32 hours old | (i) Schizont and rosette 44—48 hours old |



Fig. 1. *P. reax* trophozoites during quinac therapy.

(1)-(6) from patient TOL; (7)-(11) from patient GR1.

- | | | |
|--|--|--|
| 1a) ring stage before therapy | 2a) Maximum growth observed in 54-66 hours | 3a) Amastigotes and early schizont 8-10 hours after first therapy |
| Amastigotes 24 hours after first therapy | 2b) Half grown amastigotes before therapy | 3b) Unexcysted early schizonts 20-24 hours after first therapy |
| Amastigotes 24 hours after first therapy | 2c) Amastigotes -6 hours after first therapy | 3c) Intermittent schizont and amastigotes 12 hours after first therapy |
| 4a) Maximum growth observed in 64 hours | | |

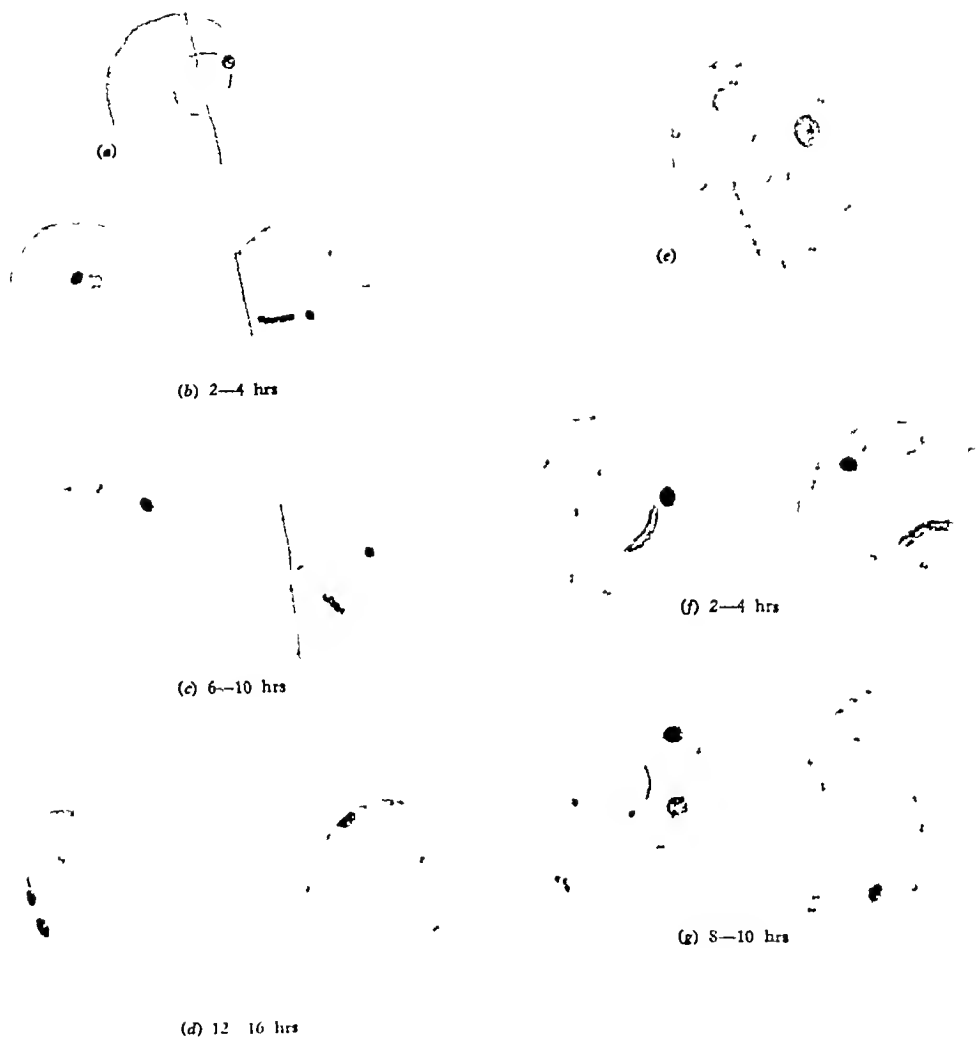


FIG 3 *P. vivax* trophozoites during atebirin therapy
From patient SLA

- (a) Young ring before therapy
- (b) Rings and amoeboids 2—4 hours after first therapy
- (c) Amoeboids 6—10 hours after first therapy

- (d) Amoeboids 12—16 hours after first therapy
- (e) Half grown amoeboid before therapy
- (f) Amoeboids 2—4 hours after first therapy
- (g) Amoeboids 8—10 hours after first therapy

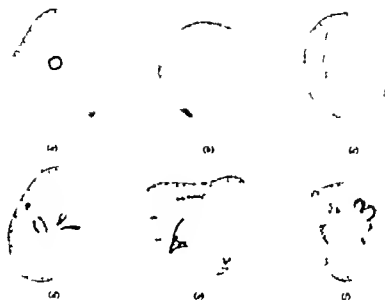


FIG. 4. *P. vivax* trophoblastic during plasmasma therapy
From patient BFL

(a)–(c) Stages in the degeneration of young
trophoblasts

(d)–(f) Stages in the degeneration of large
trophoblasts

After 6 hours practically all the rings were abnormal. No growth had taken place, and many rings were reduced to a short band of cytoplasm and chromatin pressed against the periphery of the red cell (Fig 3 c). These resembled the marginal forms seen during quinine therapy. All schizonts and preschizonts were abnormal, their nuclei being large and diffuse, and pigment prematurely clumped or extruded from the cytoplasm. After 8 hours some half-grown amoeboids were seen in which the pigment had been extruded from the cytoplasm, while in others the process appeared to have gone a stage further, with the extrusion of the pigment from the red cell (Fig 3 g). At 16 hours the young rings were represented by marginal forms, or pale wisps of cytoplasm detached from their nuclei (Fig 3 d).

The count dropped steadily, both large and small parasites being rapidly removed from the circulation. Many parasites were apparently extruded from the red cells, as cells without parasites, but containing Schüffner's dots, were frequently seen, particularly in a film taken 14 hours after beginning therapy.

Gametocytes were rarely observed until about the 24th hour, when they began to form an appreciable part of the total count. The last asexual parasites were seen in films made at the 30th hour, and consisted of remnants of half-grown amoeboids. After this period only gametocytes were seen. The results are set out in Table I.

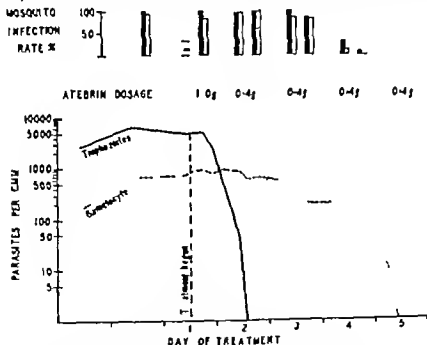
TABLE I
PARASITE COUNTS DURING ATEBRIN THERAPY Patient SLA

Day of therapy	Hours after first dose	Parasite counts per c.mm				Total gametocytes
		Rings	Half-grown amoeboids	Large trophozoites	Schizonts	Total trophozoites
1	0	4,040	840	200	80	5,160
	2	5,500	640	280	—	6,420
	4	2,140	360	80	—	2,580
	6	2,060	150	150	—	2,360
	8	840	100	70	—	1,020
	10	980	—	200	—	1,180
	12	520	—	160	—	680
	14	500	—	110	—	610
	16	—	—	—	—	180
	18	—	—	—	—	120
2	10	160	—	20	—	140
	18	100	—	40	—	80
	20	100	—	00	—	64
	22	20	—	34	—	30
	24	30	—	5	—	4
	26	25	—	8	—	8
	28	4	—	—	—	—
	30	—	—	—	—	—
	32	—	—	—	—	—
	34	—	—	—	—	—
3	48	—	—	—	—	—
4	72	—	—	—	—	—
5	90	—	—	—	—	—

(2) HEA. Before treatment began, there were present 4,200 trophozoites per c.mm.; 11 per cent. were rings, 67 per cent. young amoeboids and 22 per cent. schizonts.

Four hours after the first dose no increase in size in the amoeboids were noted, but all forms were still present, and the count had increased slightly due to the asexuals which was occurring when treatment was instituted. Eight hours after the first dose, no parachizonts nor schizonts were found, and the count was beginning to fall. The young amoeboids had ceased to grow and many were disintegrating—they formed 76 per cent. of the trophozoites, the rest (24 per cent.) being rings. By the eleventh hour most of the trophozoites present showed signs of degeneration, and the count had decreased by about 50 per cent. No new rings appeared and the blood was free from asexual forms 28 hours after the first dose of atabrin.

While the blood was being cleared of trophozoites during the first day of therapy gametocytes increased in numbers and, although non-infective for no apparent reason immediately before therapy was started, they matured normally and then remained highly infective up to the evening of the third day of treatment. Mosquitoes were still infected on the fourth day but both numbers and infectivity of the gametocytes were falling rapidly (Graph 3). Throughout treatment, the percentage of male gametocytes fell steadily from 46 per cent. on the first day to 33 per cent. on the second day 23 per cent. on the third day and 8 per cent. on the fourth day of treatment—none was recognized on the fifth day.



GRAPH 3. Parasite densities and mosquito infection rates in HEA on atabrin therapy. Histograms as in Graph 1.

(b) Partial Treatment

No volunteers on routine atabrin suppression showed macroscopically detectable numbers of *P. vivax* in the circulation (FAIRLEY 1945). However the effect of giving small doses of atabrin, when trophozoite and gametocyte

waves were already developed, was observed in one volunteer, STO, who was given gramme 0.05 on 2 alternate days at the height of his attack, followed after an interval of 2 days by gramme 0.05 daily for 3 days. Trophozoites decreased steadily during the period of partial atebirin therapy, but gametocytes increased from 700 per c mm to 1,400 per c mm during the same period. Mosquitoes were fed on six occasions, and practically all batches were 100 per cent infected. It appeared that low concentrations of atebirin, sufficient to destroy the majority of the trophozoites, were without effect on the developing gametocytes.

PLASMOQUINE

(a) Morphology

The effect of plasmoquine on trophozoites of *P. vivax* was observed in two patients, who were given plasmoquine mg 30 daily for 5 days. Both these men had trophozoite-induced vivax malaria, and were suffering from a quotidian fever. Throughout these experiments the dosage of plasmoquine is expressed as plasmoquine base, mg 10 of plasmoquine base being contained in mg 20 of plasmoquine naphthoate.

Schizogony occurred daily on the first 4 days of therapy in one patient, BEL, as either mature schizonts or young rings were observed on each day. On the fifth day of therapy, only amoeboids and preschizonts were observed, and the count fell for a further 48 hours. Then, however, a fresh crop of young rings appeared in the blood, and the count began to increase again. In the other patient, no schizogony was observed during treatment, and the count fell to submicroscopic levels for 4 days, before commencing to increase again.

Similar morphological changes were observed in both patients, and were as follows —

- (1) *Rings* — Some contracted into a small solid lump, but more usually the vacuole increased in size, until the parasite was a mere rim of cytoplasm stretched thinly around its periphery. The nucleus was similarly thinned and stretched, and was recognizable as a dark reddish section of the contour of the parasite.
- (2) *Young Amoeboids* — The chromatin appeared in many as a thin, irregular line often staining very darkly, in others it was vacuolated (Fig 4 a), or had separated from the cytoplasm (Fig 4 b). In many parasites the chromatin fragments had disappeared, either by extrusion or solution, and red cells containing pale, ragged fragments of cytoplasm without nuclei were commonly seen (Fig 4 c). The cytoplasm was often pale, with vague, irregular edges, suggesting that it was dissolving, as it were, within the substance of the red cell. In some this process had been carried so far that only nuclear remnants, with perhaps a vague suggestion of cytoplasm, remained in a red cell with Schüffner's dots.
- (3) *Large Parasites* — When amoeboids, preschizonts and schizonts were affected, the picture was fundamentally the same — a gradual disintegration and disappearance of the parasite, modified only by the fact that there was pigment as well as more cytoplasm, so that complete disappearance was unlikely. The cytoplasm of these large parasites stained a dull blue, and the pigment usually appeared in a few large granules, which were sometimes extruded from the parasite into the red cell. The nucleus appeared broken into ill-defined masses, merging with the cytoplasm, or sometimes extruded from it (Fig 4 d-f). Those individual parasites, which were not killed outright by the drug, grew, but tended to round up and divide before reaching full size, so that very small schizonts were formed.

and these produced few merozoites. Mature schizonts (i.e. rosettes) with seven and nine merozoites were seen 30 hours after the first dose of plasmoquine and at 53 hours, some were found with three, four and eight merozoites.

(4) *Gametocytes*.—W could not satisfactorily study the morphological changes in the gametocytes, as they were extremely scanty in both patients, but at least one normal male and one normal female gametocyte were seen 20 hours after commencing therapy.

(b) *Infectivity of Gametocytes*.

A single dose of plasmoquine mg 10 had produced a remarkable effect on *P. falciparum* gametocytes, causing them to lose their infectivity in about 15 hours and to disappear from the blood stream in 3 or 4 days. Its effect was tested on one patient, ROW who had numerous *P. vivax* gametocytes in the blood. There was a decrease in the heaviness of the gut infection in mosquitoes fed on him 18 hours after treatment, but they were still becoming infected after 30 hours. Exflagellation of male gametocytes was observed, but no infected mosquitoes were obtained at 42 hours. It would be inadmissible, however to attribute this solely to the drug given, as sudden changes in infectivity were frequently observed, unrelated in any way to treatment.

The percentage of cysts dying and becoming chutinizd increased from batch to batch, but sufficient developed normally to produce fairly heavy gland infections in all batches of mosquitoes fed from 8 to 30 hours after plasmoquine. This is in strong contrast to what occurred in *falciparum* infections, in which any cysts formed in mosquitoes fed at 15 hours died, and no infection at all occurred at 21 to 25 hours.

Numerous trophozoites were present in this individual, and new gametocytes were probably coming into the circulation continually. These new gametocytes either escaped the action of the drug in some way or *vivax* gametocytes are not as susceptible to plasmoquine as are *falciparum* gametocytes.

The observations are set out in Table II.

TABLE II
EFFECT OF PLASMOQUINE 10 MG. NUMBERS AND INFECTIVITY OF *P. VIVAX* GAMETOCTES.
Patient ROW

Time in relation to drug	Trophozoites per c.mm.	Gametocytes per c.mm.	Gut infection ()	Average number cysts per gut.	Sporozoite rate (%)
— 4 hrs	10 600	900	100	110	100
— ½ hr	20 900	770	100	90	100
6 hrs.		650	100	160	100
12		610	100	74	100
1	23 000	700	91	14	91
4		720	100	14	100
— 30		460	92	10	1
4	17 300	420	—	—	—

It would have been interesting to repeat this experiment using larger doses of plasmoquine, but this was impracticable owing to pressure of other work.

DISCUSSION

From our own observations, and from the evidence produced by other workers, it seems probable that quinine and atabrin act in an essentially similar way, and that their action is confined to stages which are metabolizing haemoglobin. There are several significant facts in support of this hypothesis.

(1) The changes we observed were confined to growing, erythrocytic stages of the parasite. Full-grown gametocytes are unharmed by the drugs, and can develop normally in the mosquito. We do not know, however, what happens to schizonts, which come under the influence of the drugs during the brief period between cessation of growth and sporulation, some apparently may complete schizogony (cf GRI, p 446).

(2) Pre-erythrocytic forms are able to multiply normally, subinoculation experiments showing clearly that the first generation of erythrocytic forms invades the blood stream at exactly the same time in volunteers on quinine and atabrin suppression as in controls (FAIRLEY *et al*, 1947).

(3) In bird malaria exo-erythrocytic forms are unharmed by these drugs. Chicks infected with *P. gallinaceum* may be cured of the blood infection, only to die later from the invasion of endothelial cells in brain capillaries by e-e forms.

(4) The exposure of erythrocytic *P. lophurae* to these drugs *in vitro* at low temperature does not produce morphological changes in the parasites (HEWITT and RICHARDSON, 1943), and it is extremely unlikely that any growth could occur under the conditions of the experiment.

(5) The findings with *P. vivax* hold equally well with *P. falciparum*, except that *P. falciparum* gametocytes can complete their growth in the red cell in the presence of atabrin in sufficient concentration to destroy asexual forms. They show, however, evidence of disturbance of haemoglobin metabolism, since many are unpigmented (SINTON, 1938).

Plasmoquine, on the other hand, affects all stages, irrespective of whether they are metabolizing haemoglobin or not, and irrespective of growth. Gametocytes are affected, those of *P. falciparum* being peculiarly susceptible. Pre-erythrocytic stages are also affected, subinoculations from volunteers taking mg 80 daily for the first 5 or 8 days after infection being negative at the end of the normal pre-patent period (FAIRLEY *et al*, 1947). *P. lophurae* showed morphological changes when exposed to plasmoquine *in vitro* at 6° C, and, when this blood was inoculated into a new bird, there was great delay in the incubation period and changes in the parasitological response which suggested that only a minute fraction of the parasites injected were viable (HEWITT and RICHARDSON, 1943).

In addition to the modes of action outlined here, two others have been observed recently. Paludrine has been shown to act upon the dividing nucleus of the full-grown trophozoite, so that merozoites are not formed (BLACK, 1946, MACKERRAS and ERCOLE, 1947), whilst in experiments with *P. falciparum in vitro* sulphadiazine has been shown to act only on the divided schizont (BLACK, 1946). The metabolic processes of malarial parasites are obviously complex, and different drugs obviously affect them in quite different ways. It seems probable that those, which affect the nucleus, are the most likely to be effective against all phases of the parasite.

SUMMARY AND CONCLUSIONS

1 The action of quinine and atabrin on the erythrocytic cycle of *P. vitex* is direct, and is evident within 4 hours of oral administration of the drug. Growth is retarded or stopped, and morphological signs of degeneration appear in all growing stages.

2 Parasites of a single brood vary in their susceptibility to quinine, some being quickly destroyed, while others continue to grow slowly for many hours. *P. vitex* trophozoites of all ages are more susceptible to the action of atabrin than of quinine.

3 These drugs appear to act by inhibiting the metabolism of haemoglobin. They have no action on parasites whose growth is independent of this protein, nor on gametocytes which have already completed their growth.

4 Plasmoquine acts more as a general protoplasmic poison, its action being independent of stage of growth or substrate used for metabolism. *P. vitex* gametocytes appear to be less sensitive to plasmoquine than are those of *P. falciparum*.

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OBSERVATIONS ON THE ACTION OF QUININE,
ATEBRIN AND PLASMOQUINE ON THE GAMETOCYTES
OF *PLASMODIUM FALCIPARUM*

BY
M J MACKERRAS
AND
Q A FRCOLE

It is well known that *Plasmodium falciparum* gametocytes (crescents) are extremely resistant to most drugs, plasmoquine alone having a dramatic effect upon them. We had the opportunity of extending these observations while members of the L H Q Medical Research Unit, Cairns, Queensland, and we wish to thank Professor N HAMILTON FAIRLEY, formerly Director of the Unit, for permission to publish these notes.

We were able to collect data showing the relative effect of quinine and atebtrin on gametocyte production. We were also able to confirm SINTON's observations on the production of non-pigmented gametocytes and their infectivity to mosquitoes. All the observations were made on Melanesian strains of *P. falciparum* in highly susceptible white hosts. The mosquitoes used belonged to the natural vector species *Anopheles punctulatus punctulatus* Dönitz.

QUININE

It is well recognized that therapeutic doses of quinine, given when gametocytes are already present in the blood, are without effect on their number, morphology or infectivity to mosquitoes, and our experience merely confirms that of other workers. We found, however, that quinine had a very deleterious effect upon young gametocytes, or their precursors. Volunteers on inadequate suppressive doses, or patients either partially or fully treated with quinine, never exhibited high gametocyte waves comparable with those seen in patients having partial atebtrin or paludrine therapy.

In Table I the maximum gametocyte counts of patients inadequately suppressed by quinine are set out in relation to the maximum trophozoite counts and the number of days trophozoites had been present before therapy began. Only those patients are included, in whom gametocytes should have appeared before any plasmoquine was given. It will be seen that only one out of seven showed any gametocytes, and this patient had had trophozoites present for the longest period (14 days) when treatment began.

Mean plasma quinine levels for the group on gramine 0.33 daily ranged from 0.35 (minimum) to 2.15 (maximum) mg. per l., and for the group on gramine 0.66 from 0.65 (minimum) to 4.09 (maximum) mg. per l.

TABLE I.
GAMETOCYTE PRODUCTION IN PATIENTS ON INADEQUATE QUININE THERAPY, EVENTUALLY TREATED WITH Q.A.P.†

Name	Suppression quinine daily dose (gramme).	Days trophozoites present when treatment started	Maximum trophozoites per c.mm.	Maximum gametocytes per c.mm.
STE*	0.33	5	49,000	0
DEL	0.33	7	17,800	0
GOD	0.33	8	64,000	0
REC	0.33	10	10,400	0
KEL	0.66	11	31,400	0
LAW	0.66	13	74,000	0
BOV	0.66	14	38,000	75

* Given intramuscular injections of quinine gramine 0.33 or gramine 0.66 on 2 alternate days followed by Q.A.P.

† Q.A.P. = Standard course of treatment, namely quinine gramine 2.0 daily for 3 days, atabrin in decreasing doses from gramine 0.6 to 0.3 daily for 5 days, quinine gramine 1.0 and plasmoquine gramine 0.03 daily for 5 days.

In Table II details of the gametocyte production in patients treated with quinine are set out. If treatment was begun by giving small doses of quinine (gramine 0.33 or gramine 0.66) daily for a few days before commencing full therapy gametocytes appeared more frequently but the counts were low although the trophozoites sometimes reached densities in excess of 100,000 per c.mm.

These figures may be compared with those for a group of volunteers given partial therapy with atabrin, followed by various modifications of the standard course of treatment (Table III). This group is comparable with Group II in Table II i.e. they had previous exposure to *P. falciparum* without a clinical attack.

TABLE II
GAMETOCYTE PRODUCTION IN EXPERIMENTAL SPOROZOITE-INDUCED MALARIA FULLY OR
PARTIALLY TREATED WITH QUININE

Group	Name	Days trophozoites present when first treated	Maximum trophozoites per c mm	Maximum gametocytes per c mm	Type of therapy used	
					Partial	Complete
I	AAN	6	84,000	8	—	Q A P
	AUS	6	108,000	0	—	Q A
	ARM	7	315,000	0	—	Q A P
	BAR	7	240,000	0	—	Q A P
	HAY	7	90,000	0	—	Q A P
	STE	8	32,000	0	—	Q A
	COW	6	290,000	4	Quinine	Q A P
	DEL	9	147,000	50	"	Q A P
	GRE	6	27,000	0	"	—
	GRE	6	16,900	0	"	Q A P
	WAR	8	306,000	3	"	Q A P
II	BUN	8	273,000	1	—	Q A
	LAW	5	87,000	0	—	Q A
	KEN	6	156,000	10	—	Q A P

Group I—First attacks in volunteers not previously exposed to malaria

Group II—First attacks in volunteers previously exposed to *P. falciparum*, suppressed and cured of first infection, and then reinfected after intervals of 3 to 4 months

It will be seen that the numbers of gametocytes produced, when quinine was used for partial or full therapy, were very much lower than those observed when partial treatment with atabrin was used. They were also lower than when larger doses of atabrin (gramme 0.5 daily for 3 days) were used, or when paludrine (gramme 0.3 daily for 10 days) was given (MACKERRAS and ERCOLE, 1947). This suggests that quinine prevents the growth of young gametocytes in the same way that it inhibits the growth of trophozoites *in vitro* (BLACK, 1946). Our results thus confirm those of SINTON (1938), who found that quinine was more effective than atabrin in reducing the number of potential gametocyte carriers.

SINTON's results and our own may appear, at first, at variance with those of other workers who have reported high and prolonged gametocyte waves after quinine therapy. FIELD (1938), for example, gives details of two cases where this occurred. However, he was working in an endemic area, and his patients were Indian or Chinese labourers who might have had previous infections.

One of his cases was probably primary, but treatment was not given until the tenth day of the attack, and gametocytes appeared on the following day. In the other patient treatment was given on the seventh day, but as gametocytes appeared next day, trophozoites had probably been present longer than 7 days. At all events, in each of his cases gametocytosis was apparently well advanced before quinine was exhibited, whereas, in the majority of our patients treatment began when few, if any, gametocytes were fully grown.

TABLE III.

GAMETOCYTE PRODUCTION IN EXPERIMENTAL SPOOROZONTE-INDUCED MALARIA PARTIALLY TREATED WITH ATEBRIN.

Name.	Days trophozoites present when first treated.	Maximum trophozoites per c.mm.	Maximum gametocytes per c.mm.	Number of days of partial therapy	Type of therapy used.	
					Partial.	Complete.
CAR	3	164,000	4	4	Atebrin	Q A P
EGA	8	360,000	180	3		Q A.
ELL	8	124,000	140	3		Q A.
GOW	7	280,000	140	3		Q A.
DRI	5	73,000	280	7		Q
LAI	4	62,000	280	7		Q
NEI	5	103,000	140	7		Q
FUG	3	64,000	250	7		A.
WHI	1	85,000	790	1		A.
STA	2	50,000	360	8		A.

Explanation of abbreviations used —

Q.A.P. = the standard course of treatment, see p. 456

Q.A. = the first 2 days of standard course.

Q = the first 3 days of standard course followed by atebria gramine 0.1 maintenance.

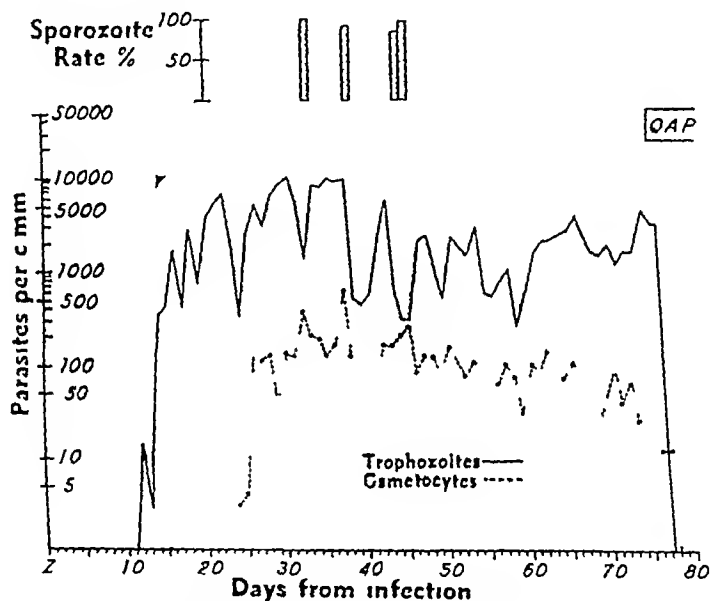
A. = the atebria portion of Q.A.P. followed by gramine 0.1 maintenance.

ATEBRIN.

In sporozoite-induced falciparum malaria, gametocytes usually appeared 9 to 12 days after the first trophozoites were detected, and a similar interval occurred between the peaks of the trophozoite and gametocyte waves. This long interval between the appearance of trophozoites and of gametocytes is usually interpreted to mean that falciparum gametocytes require 10 days or so to develop. It may be less than this as gametocytes appeared earlier in trophozoite induced malaria and the peak of the gametocyte wave usually occurred about 8 days after the peak of the trophozoite wave. However it seems evident that they require a much longer period to mature than do schizonts, and that the effect of therapy upon the developing gametocytes will be observed not at the time of treatment, but some 7 to 14 days later.

(a) Suppressive doses

Gametocytes as well as trophozoites sometimes appeared in volunteers infected with falciparum malaria which was incompletely suppressed by small doses of atebirin. The gametocytes were usually scanty, but in one volunteer (FOR) on atebirin gramme 0.3 per week, who remained ambulatory, gametocytes were present for over 50 days, and counts ranging from three to 530 per c mm were recorded. Mosquitoes were fed on him on four different days, and well infected batches were secured on each occasion. During the period of observation this volunteer's plasma atebirin level averaged 11.3 microgrammes per l.



GRAPH 1—Parasite densities and infection rates in mosquitoes in FOR on inadequate atebirin suppression

(b) Therapeutic doses given when gametocytes were already present

Large doses of atebirin given when gametocytes were present in the blood did not influence their numbers, morphology, nor infectivity to mosquitoes. One patient, CDS, was allowed to remain infected for a considerable time, the severity of his attacks being controlled by intermittent small doses of atebirin. Full treatment was begun on the 50th day, just at the peak of his second gametocyte wave. Mosquitoes were fed every second day during the period of quinine and atebirin therapy, and the results are set out in Table IV. The decrease in infectivity which occurred was no greater than that usually observed in patients, who were receiving partial therapy.

c) *Therapeutic doses given before gametocytes were present in blood*

If atabrin grammae 1.0 to 3.4 were given during the acute stage of the infection, when only trophozoites were present it usually terminated the attack, but seldom prevented the appearance of gametocytes 5 or 6 days later. This sequence was observed in about 50 cases. These gametocytes appeared in densities which could be correlated, on the whole, with the trophozoite densities previously observed, and with the degree of immunity possessed by the patient.

TABLE IV

INFECTIONS IN MOSQUITOES DURING OUTDOOR AND ATABRIN THERAPY. PATIENT CDS.

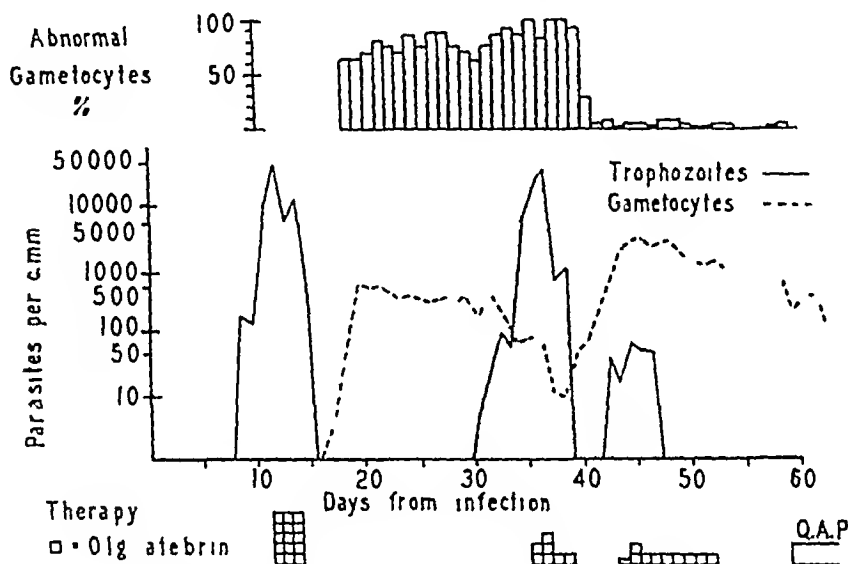
Day after infection.	Drug given.	Gametocytes per cmm.	A stage number cysts per gut.	Sporozoite rate (%)	Gland infection.
50	Quinine grammae 2	3,000	73	100	Heavy
51		2,740			
52		3,500	69	100	
53	Atabrin 0.6	3,140			
54		0.8	2,400	22	100
55		0.4	2,840		
56		0.3	1,900	10	91
57		0	1,600		
58	—	1,200	3	59	Medium

Morphologically, however many of the gametocytes were abnormal in one respect, namely in the amount of pigment present. The proportion affected varied enormously and sometimes reached 100 per cent. It was usual to see every grade from completely unpigmented to fully pigmented gametocytes in the same patient, but the majority contained at least a few fine granules of pigment.

In other respects these gametocytes were normal. Their measurements fell within the normal range, cytoplasm and nucleus appeared normal, and they proved capable of infecting mosquitoes.

The prevalence of unpigmented and slightly pigmented forms could be correlated with the amount of atabrin given to control the acute attack, and indirectly with the state of immunity of the patient, since the more immunity a patient possessed, the less atabrin he required to control the trophozoite densities and the fewer were the abnormal gametocytes produced. The spacing of the doses also influenced the result, as two or three large doses on consecutive days had more effect upon the gametocytes developing at that time than the same total amount spread over a long period. The sequence of events in a typical case, FLE, is shown in Graph 2.

Observations were made on gametocyte production in patients infected with relatively atebtrin-resistant strains of *P. falciparum* from the Wewak area of New Guinea (FAIRLEY *et al.*, 1946). Although these patients usually required atebtrin gramme 2.0 to 3.0 to control the trophozoite wave, the proportion of unpigmented or lightly pigmented forms in the subsequent gametocyte wave seldom exceed 50 per cent. This was in marked contrast to the results with "normal" strains from South-Eastern New Guinea. In these the trophozoite wave could usually be terminated by atebtrin gramme 1.5, and the proportion of abnormal gametocytes was usually in excess of 50 per cent (Graph 2).



GRAPH 2—Parasite densities and incidence of abnormal gametocytes in relation to therapy in FLE, treated with atebtrin

The trophozoites of relatively resistant strains are able to grow and multiply in the presence of a considerable concentration of atebtrin, apparently metabolizing haemoglobin in the normal way. It is therefore not surprising that the gametocytes growing in the same environment should also metabolize haemoglobin, and produce pigment, as they would without atebtrin. With atebtrin sensitive strains, on the other hand, the trophozoites are unable to grow in the presence of a certain concentration of atebtrin. Gametocytes, however, succeed in growing to maturity, but not in producing the usual amount of pigment.

SINTON (1938) suggested that the pigment was formed, but was extruded by the parasite under the influence of atebtrin. This may be the correct explanation, but we have not seen evidence of extrusion in the many preparations

from the peripheral blood which we examined. In all other respects, e.g. relationship of dosage to the production of unpigmented forms, and behaviour of these forms in the mosquito, our findings agree closely with his.

The behaviour of these forms was studied in smears made from blood in the stomach of the mosquito at intervals after feeding. Exflagellation of unpigmented male gametocytes, and fertilization of unpigmented female gametocytes were observed. The unpigmented zygotes developed at a normal rate, forming unpigmented vermucules, which in turn gave rise to unpigmented cysts. These cysts grew normally and there is no reason to suppose that they failed to produce sporozoites. This could not be proved, however since pigment cannot be readily detected if at all, in a sporulating cyst.

PLASMOQUINE

The remarkable effect of small doses of plasmoquine on *P. falciparum* gametocytes has been recorded by many observers (BARKER *et al.*, 1929; JERACK and GIOVANOLA 1933, etc.) In preliminary experiments, we found that mosquitoes fed 3 hours after the administration of a single dose (mg. 10) became infected, but that after 15 hours no infection was recorded. We then set up a more elaborate experiment, choosing a highly infectious donor and feeding mosquitoes at frequent intervals during the first 15 hours after a single dose of plasmoquine mg. 10. The results are set out in Table V.

TABLE V

EFFECT OF PLASMOQUINE MG. 10 ON NUMBER AND EFFECTIVITY OF *P. falciparum* GAMETOCYTES.

Hours after drug.	Gametocytes per count.	Exflagellation	Vermucule formation	Gut infection (%)	Average number cysts per gut	Sporozoite rate (%)	Gland infection
- ½ h	870		Normal	100	24	100	Heavy
1				100	36	7	
- 2 hrs				79	30	64	Medium
4				83	29	73	
9			Delayed	7	8	33	Light
12				51	6	24	
15				8	1	Nd	—
+ 3	970		—	Nd	Nd		
+ 33	710		—				
+ 49	310						
64	14						
7	32						
- 94	12						

It will be seen that the sporozoite rate began to diminish during the first 6 hours, although large numbers of cysts were formed. At 9 hours there was an abrupt fall in the number of cysts per gut, and many of these failed to develop, the sporozoite rate falling sharply. After 15 hours very few cysts developed, and no gland infections were detected. After 25 hours the gametocyte count was still high, but no mosquitoes became infected, although exflagellation was observed. The count then fell steadily and was reduced to a negligible figure 4 days after the drug had been given, and the few gametocytes then found were degenerating.

CONCLUSIONS

1 Mature gametocytes are unharmed by quinine and atabrin, but are peculiarly susceptible to plasmoquine, a minute dose (mg 10) being sufficient to render them non-infectious to mosquitoes within 15 hours, and to cause their disappearance from the blood in 3 or 4 days.

2 In the dosages employed, quinine inhibits the growth of young gametocytes, whereas atabrin affects their metabolism in such a way that they appear unpigmented or with only minute amounts of pigment. These unpigmented forms are capable of infecting mosquitoes.

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STUDIES ON A WEST AFRICAN STRAIN OF *PLASMODIUM FALCIPARUM*

II THE EFFICACY OF PALUDRINE (PROGUANIL) AS A THERAPEUTIC AGENT

BY

G COVELL, W D NICOL,
P G SHUTE AND M MARYON

In the first of this series of papers an account was given of prophylactic trials with paludrine against infections with a strain of *Plasmodium falciparum* obtained from an African child resident in Lagos, Nigeria, West Africa. The present paper deals with therapeutic trials against infections with the same strain of parasite, which has been maintained at Horton since November, 1947, by successive blood and mosquito inoculation. None of the subjects through whom the strain has been passaged have received paludrine except the second in the series, who was given mg 300 of the drug in a single dose on two successive days. The mosquitoes used for transmission were *Anopheles stephensi* (Type), a colony of which was established at the Ministry of Health's Malaria Laboratory, Horton, during the winter of 1947-48, from specimens imported by air from India*. As in the prophylactic trials previously reported, the subjects of the

* We wish to acknowledge the assistance of Lt-Col JASWANT SINGH, Director, Malaria Institute of India, in establishing this colony

trials were patients in Horton Hospital, who had undergone malaria therapy for neurosyphilis with the Madagascar strain of *Plasmodium vivax* or in some cases, with European strains of *P. falciparum*, 9 or more years previously but had not been exposed to malarial infection of any kind during the intervening period. Eighteen of the patients had taken part in the prophylactic trials, but none had developed overt malarial attacks. (COVELL, NEOL, SUTER and MARTON 1949).

MACGRAITH and his colleagues at the Liverpool School of Tropical Medicine, who were the first to use paludrine in the treatment of human malaria, published records relating to 22 patients naturally infected with West African strains of *P. falciparum* 16 of whom were considered to be suffering from primary attacks. Specific treatment was withheld for several days in order to ensure that spontaneous cure of the attack was not occurring, except in cases where the condition of the patient was too serious to allow of this procedure. The dosage of paludrine administered varied from mg 50 to mg 600 twice daily over a period of 14 days. The clinical response was satisfactory in all cases, and was as rapid in those receiving mg 50 of the drug twice daily as in those who were given a dosage ten times as great. It was not possible to determine the radical cure rate, owing to difficulties in follow up (MACGRAITH ADAMS *et al.*, 1945).

FAIRLEY *et al.* (1946) reported radical cure in 41 out of 41 natural, and in 46 out of 47 experimentally induced sporozoite infections with New Guinea strains of *P. falciparum* treated with a 10 days course of paludrine mg. 100 thrice daily. In most cases there was rapid clearance of asexual parasites from the peripheral blood, but though the overt attack was readily controlled, even by very low dosage the clinical response was described as "not rapid." Gametocytes were not destroyed in the peripheral blood, but were rendered non infective to mosquitoes for a variable period depending on the size of the dose administered.

A number of therapeutic trials with paludrine have been carried out in India and in Malaya, with generally favourable results. The drug has proved particularly useful among labour forces and village populations, where a single dose treatment of mg 300 has been widely adopted. The great majority of the subjects treated have, however been persons possessing some degree of tolerance as the result of previous infections, and there is little evidence from either country as regards the radical cure rate. CHAUDHURI (1948) has drawn attention to the existence in India of strains of *P. falciparum* which are not radically cured by a dosage of paludrine mg 300 daily for 10 days, citing the case of a patient who experienced a second attack 9 days after the completion of treatment and a third 11 days later. Other reports from practitioners in India and Malaya indicate that for the treatment of falciparum infections in non-immune persons, paludrine unadjuvanted is not entirely adequate as a therapeutic agent in the dosage originally recommended.

Comparatively little data have hitherto been published regarding the treatment of malaria in Europe with paludrine. BETTINI (1948) has, however, recorded details relating to 94 cases of falciparum infections treated at Posada, Sardinia, with paludrine mg 100 thrice daily for 10 days, followed by a weekly dose of mg 100 for 3 months. The clinical response and clearance of parasites from the peripheral blood was satisfactory, but in 17 of the cases relapses* occurred within this period.

Widely divergent accounts regarding the efficacy of paludrine in the treatment of malaria have emanated from different parts of East, West and Central Africa. Some of these have been favourable, whilst in others it has been alleged that the drug has proved unsatisfactory in the treatment of falciparum infections as regards both clinical response and radical cure. The most frequent criticism has been that owing to its slowness of action, serious symptoms have persisted in many cases for 3 or 4 days, sometimes longer, necessitating reinforcement of the treatment with mepacrine or quinine. In the Belgian Congo, however, VAN RIEL (1948) has reported that the clinical response in 156 cases of falciparum malaria in African labourers treated with paludrine mg 100 thrice daily was of the same order as that achieved by mepacrine or quinine. The radical cure rate was not stated.

The investigations recorded below were undertaken with the object of clearing up some of the points at issue regarding the treatment of infections with African strains of *P. falciparum* with paludrine.

DETAILS OF THERAPEUTIC TRIALS

Thirty-one patients were arranged in groups as shown in Table I. Preliminary trials had shown that a dosage of paludrine mg 300 daily for 7 days was inadequate for the production of radical cure. Groups I and II were planned to ascertain whether doubling the period over which the dose was given or doubling the daily dose over the same period would have any effect on the course of the disease, Groups III and IV to test the value of reinforcement of a paludrine course with mepacrine or quinine given on the first day of treatment, Group V, to compare the effects of the above regimes with that of quinine only, and Group VI to study the comparative effect of a single day's treatment with each of the three drugs under trial.

Drug administration was under strict supervision throughout, and a record was maintained of every dose given, each entry bearing the signatures of two of the nursing staff. Treatment was commenced in all cases as soon as the temperature reached 100° F, provided that parasites had also been seen in the peripheral blood (thick smear).

* Throughout this paper the term "relapse" has been applied to any recrudescence of fever with demonstrable parasitaemia subsequent to recovery from the primary attack.

Each patient was infected by intravenous inoculation of a suspension of the salivary glands of two infected mosquitoes, by the technique described by SMITH (1937). The sporozoite count in respect of each infection, which represents the minimum number of sporozoites actually injected, is shown in Table II together with the date of infection and the length of the incubation and pre-patent periods.

All the patients received their infection from the same batch of mosquitoes. Those in the first three groups were infected within 24 hours after the first detection of sporozoites in the salivary glands, the remainder 4 days later. The

TABLE I

Group	Number in each group	System of treatment
I	5	Paludrine mg. 300 once daily for 14 days
II	5	200 m.l.c. 7
III	5	300 10 plus mepacrine mg. 300 t.d.s. on the first day only
IV	5	Paludrine mg. 300 m.l.c. daily for 10 days, plus quinine hydrochloride grain 10 t.d.s. on the first day only
V	5	Quinine hydrochloride grain 10 twice daily for 10 days
VI	2	10 t.d.s. one day only
	2	Mepacrine mg. 300 t.d.s. one day only
	2	Paludrine 300

mosquito infections were very heavy and as the batch was fed on sugar solution only from the time when the first oöcytes ruptured, it was to be expected that the number of sporozoites injected on the second occasion would be greater than on the first. Actually the sporozoite count showed that the members of Groups IV, V and VI received on an average three times as many sporozoites as those in the other three groups. It will be noted that the length of the pre-patent period in those inoculated on the first occasion varied from 7 to 10 days, whereas in the case of the second and more heavily infected batch of patients it was 7 days in every case. It is evident that though this period may be shortened to some extent by increasing the dose of sporozoites injected, it cannot be reduced below a certain limit, which is presumably determined by the length of time required for completion of the pre-erythrocytic phase of the parasitic cycle. Our results afford no indication that the dosage of sporozoites has any appreciable effect on the severity of the subsequent clinical attack or on the frequency of relapse.

TABLE II

Group	Subject	Date of infection	Sporozoite count	Length in days of	
				Incubation period	Pre-patent period
I	EC	13.9.48	10,000	7	7
	EM		67,500	7	8
	JT		80,830	9	9
	GW		175,000	10	8
	SB		88,300	7	7
II	RB	"	No count	7	7
	HG		101,600	11	9
	EG		10,000	8	9
	FM		98,600	7	9
	AP		101,600	7	7
III	SR	"	25,000	7	7
	TL		69,000	9	8
	WC		97,000	7	7
	ML		114,000	11	10
IV	JS	17.9.48	80,500	8	8
	DG		4,500	7	7
	HC		195,000	7	7
	VC		208,750	7	7
V	FW	"	205,000	7	7
	EC		269,000	7	7
	MB		223,250	7	7
	WE		122,500	7	7
	LH		272,000	7	7
VI	GR	"	140,000	7	7
	JF		207,500	7	7
	HB		215,000	7	7
	GB		200,000	7	7
	AG		100,000	7	7
	WH		500,000	6	7
	JK		275,000	7	7
	EM	"	140,000	8	7

RESULTS OF TREATMENT

Details in respect of Groups I to V are summarized in Table III and in Chart

Group VI—It was intended that each of the six patients in this group should receive a single day's treatment only in the first place, but in three cases this plan was modified on clinical grounds

CASES 1 AND 2 (quinine hydrochloride grain 10 t d s for 1 day only) both continued to have pyrexia throughout the third day after drug administration, and it was not considered advisable to leave them without further treatment any longer. They were therefore

given paludrine mg 300 twice daily for 10 days. The duration of pyrexia following the first dose of quinina was 92 hours in each case. Asexual parasites were present in the peripheral blood for 2 days in each case. Neither case has relapsed.

CASE 3 (Mepacrine mg 300 t.d.s. for 1 day only). Temperature fell to normal 24 hours after the first dose and except for a rise to 99.8 F on the third day and 99.0 F on the fifth, remained at or below normal for 14 days, when a second overt attack commenced. After taking single dose of paludrine mg 300 the patient refused further oral treatment so was given an intramuscular injection of mepracrine (mepacrine musonate) mg. 600 on the following day. The temperature fell to normal 40 hours later and there has been no further rise. Blood films were negative from the second day after the injection of quinacrine. The sporozoite count in respect of this patient (V.H. in Table II) was more than twice as high as any of the others.

TABLE III.

Group	Average duration of fever	Average duration of parasitaemia (asexual)	Subsequent history
I (P only)	76 hours	3.3 days	All 4 cases relapsed within 3 weeks and were treated with paludrine mg 300 twice daily for 10 days. A second relapse occurred in one case.
II (P only)	65	2.0	Four out of 8 cases relapsed within 3 weeks and were treated with paludrine mg 300 twice daily for 10 days. A second relapse occurred in 2 cases.
III (P plus M)	59	2.4	No relapse†
IV (P plus Q)	74	2.0	†
V (Q only)	85	2.0	†

/ after commencement of treatment

† Observation period 6 months

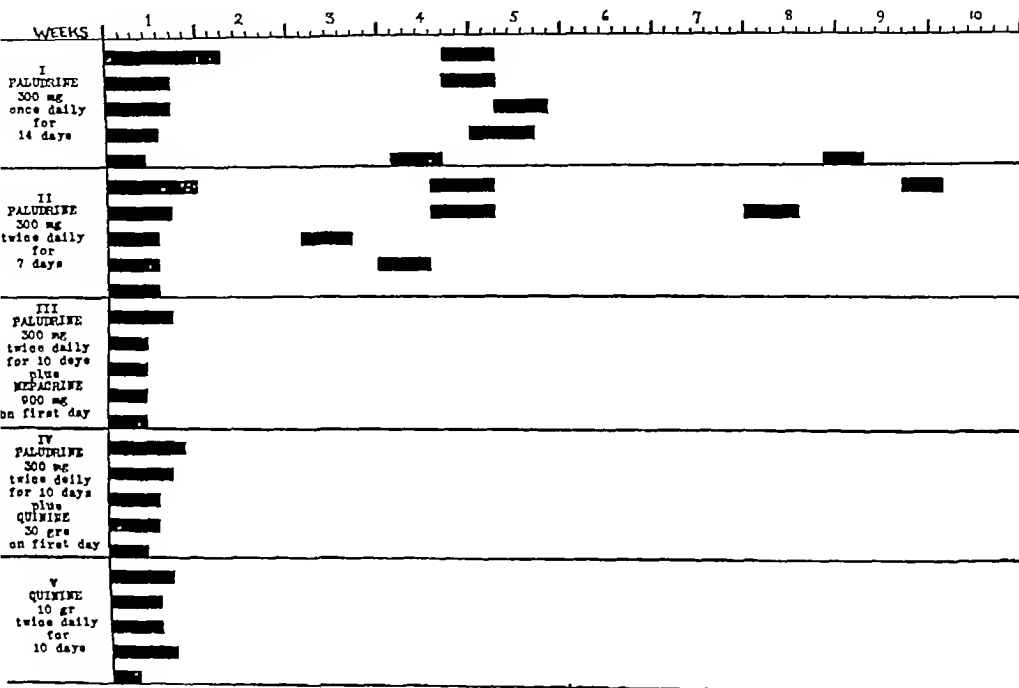
Note:—P = paludrine M = mepacrine Q = quinine.

CASE 4 (mepacrine mg. 300 t.d.s. for 1 day only). Temperature fell to normal 70 hours after the first dose but owing to the patient's previous clinical history it was not considered advisable to withhold further specific treatment. He was therefore placed on course of paludrine mg 300 twice daily for 10 days and thus should properly be considered as member of Group IV. There was rise of temperature to 99.0 F on the third day after which it continued at or below normal. Parasites were seen in the peripheral blood up to the third day after the first dose of mepacrine. There has been no relapse.

CASE 5 (paludrine mg 300 t.d.s. for 1 day only). Temperature fell to normal 68 hours after the first dose but rose to 99.0 F on the evenings of the third and fourth days. After this there was a fever-free period of 6 days when a second overt attack commenced, which was treated with paludrine mg 300 twice daily for 10 days. Blood films were negative from the third day following drug administration in the first attack, and from the second day after commencement of treatment in the second. There has been no further incident.

CASE 6 (paludrine mg 300 t d s for 1 day only) Temperature fell to normal 64 hours after the first dose, and remained at or below this figure for 14 days, when a second overt attack commenced, which was treated with paludrine mg 300 twice daily for 10 days blood films were negative from the second day after drug administration in the first attack, and from the third day after commencement of treatment in the second. There has been no further incident

The chief feature of interest in the history of Group VI was the slow clinical response following a single day's treatment with quinine as compared



NOTE —Black areas denote periods of fever in the primary attack and in relapses

with the very rapid action of a single day's treatment with mepacrine. Paludrine occupied an intermediate position in this respect. Deductions from so small a group of cases must necessarily be of limited validity.

OBSERVATIONS ON GAMETOCYTES

Except for a single female crescent seen in a thick blood smear from a patient who had been treated with paludrine mg 300 twice daily for 10 days reinforced with quinine grain 30 on the first day, no gametocytes have been observed in the peripheral blood of any patient who has not experienced a relapse. Conversely, with one exception, gametocytes have been found, often

in very large numbers, in the peripheral blood of every relapsing case. All these patients were treated for their relapse with paludrine mg. 300 twice daily for 10 days. Mosquitoes (*A. stephensi*) were allowed to feed on the two patients showing the greatest density of crescents at varying intervals after the last dose of paludrine was taken, but though ex flagellation of male gametocytes occurred regularly no vermicles were observed, nor were oöcysts seen on the stomach wall, over a period of 24 days. By this time gametocytes were no longer present in numbers sufficient to cause infection in the mosquito even had no drug been taken.

DISCUSSION

The outstanding feature of the investigations here recorded is the signal failure of paludrine, unaided, to effect a radical cure of infections with the Lagos strain of *P. falciparum*. In marked contrast with the findings of FAIRLEY and his colleagues in respect of infections with New Guinea strains of this species of parasite. In our series, 90 per cent. of the cases treated with paludrine alone relapsed within 3 weeks after completion of the course, and 33 per cent. of these relapsed a second time. whereas, in FAIRLEY's series of experimentally induced sporozoite infections, radical cure was effected in 46 cases out of 47. We do not consider that any other records of therapeutic trials with paludrine which have come to our notice are directly comparable with ours. The majority relate to patients possessing some degree of tolerance as the result of previous infection or because specific therapy has been withheld in the initial stage of the attack. For the proper evaluation of the efficacy of an antimalarial drug it is essential that its administration be commenced early in the attack, before there has been time for the building up of any substantial degree of immunity and at exactly the same stage of the disease in all cases. The dosage of sporozoites inoculated should also be as heavy as any which might be encountered in nature. The fulfilment of all these requirements is only possible under controlled experimental conditions.

It may be argued that the West African strain of *P. falciparum* used by us is naturally resistant to paludrine, but against this it should be noted that the drug proved entirely adequate as a causal prophylactic of infections with this strain, that control of the clinical attack and clearance of asexual parasites from the blood was as rapid as in the reports of most other workers and that gametocytes were rendered non-infective to mosquitoes. Had the strain been "paludrine resistant" we would have anticipated break-throughs in the prophylactic trials and failure to control the clinical attack or to clear asexual parasites from the peripheral blood in at least a proportion of the cases treated.

In FAIRLEY's series volunteers with experimentally induced virus infections were allowed to have overt malaria for several days before therapy was commenced. It is not stated whether this was also the practice with experimentally induced falciparum infections. If this was so FAIRLEY's series and ours are not strictly comparable.

It is true that the clinical response with paludrine in our series was somewhat less rapid than with mepacrine or with quinine, but this slowness of action has been remarked by a majority of those who have used paludrine for the treatment of malaria in any country where the disease is prevalent. The comparative frequency of relapse in our series of cases is less likely to be due to some peculiar quality of the strain with which we were dealing than to the fact that our patients possessed no immunity whatever at the time of infection, and that specific treatment was commenced in all cases at an early stage in the primary attack. As was pointed out by JAMES, NICOL and SHUTE (1931), the development of malarial immunity can be accelerated and the liability to relapse lessened by withholding specific treatment during the first few days of the attack. Whilst this procedure might be considered justifiable in dealing with benign tertian infections, malignant tertian malaria is far too serious a disease to warrant its adoption solely for the purpose of reducing the relapse rate. Any suggestion that the Lagos strain has become resistant to paludrine as the result of prolonged exposure to sub-therapeutic dosage can be definitely excluded.

The principal objectives in the treatment of falciparum infections are

- (i) Termination of the clinical attack with the least possible delay. In countries where malignant tertian infections are prevalent this is an essential requirement, outweighing all others in order of importance, particularly when, as frequently happens, the patient has been ill for some days before the commencement of specific treatment.
- (ii) Prevention of relapses, which may be little less dangerous to life than the primary attack.
- (iii) Sterilization of gametocytes for as long a period as possible.
- (iv) Minimum toxicity on the part of the specific drugs employed.

We have shown that, in the absence of reinforcement with mepacrine or quinine, paludrine fails to effect radical cure of infections with the Lagos strain of *P. falciparum*, and that it is comparatively slow in action as regards clinical response. On the other hand, it fulfils the other two requirements postulated above, namely, sterilization of gametocytes and a degree of toxicity lower than that of any other known antimalarial drug.

Mepacrine, when administered in the dosage now usually prescribed, *i.e.*, commencing with a "loading dose" of mg 600 to 900 on the first day or first two days of treatment and continued at mg 300 daily for 5 to 7 days, will effect a rapid termination of the clinical attack and a high radical cure rate in falciparum infections. This rapidity of action is in harmony with the observation frequently made in this laboratory that degenerative changes in the parasites of *P. vivax* and *P. malariae* (agglutination of pigment, followed later by vacuolation in the cytoplasm and disintegration of the parasite) can be detected less than half an hour after oral administration of a single dose of mg 600 of the drug (JAMES, 1934). The rapidity with which mepacrine resolves the

clinical symptoms of malaria has not in the past been fully appreciated, because until comparatively recently it has been the usual practice to limit the amount prescribed to mg 100 three daily without a preliminary loading dose. Mepacrine does not, however, sterilize gametocytes; moreover its toxic effects, though of rare occurrence, are by no means negligible. This applies particularly to the so-called mepacrine psychosis, which may be attended by symptoms of mental aberration or even of maniacal excitement. The yellow coloration of the skin, which is a common feature in mepacrine prophylaxis and may also follow a course of mepacrine therapy is an additional disadvantage.

Quinine, when administered in a daily dose of grain 20 to 30 over a period of 7 to 10 days, will like mepacrine, ensure in most cases a rapid termination of the clinical attack and a high radical cure rate. Like mepacrine it has no effect on the infectivity of gametocytes to mosquitoes, but a more serious disadvantage is its long recognized association with the precipitation of blackwater fever. FINDLAY and STEVENSON (1944) have recorded a striking reduction in the incidence of this disease among British troops stationed in West Africa following the substitution of mepacrine for quinine in the prophylaxis and treatment of falciparum infections.

We have shown that reinforcement of a 10 days course of paludrine mg. 300 twice daily with mepacrine mg. 800 given in three doses on the first day of treatment brings about rapid termination of the clinical attack and a high rate of radical cure. It has been suggested that in falciparum infections the paludrine course should be reinforced in serious cases only. But we contend that every case of falciparum malaria in non-immune persons is potentially serious, since the patient may become dangerously ill at any stage of the attack with little or no warning. We therefore recommend reinforcement of the paludrine course in all cases of falciparum malaria with mepacrine mg. 300 t.d.s. (mg. 900 in all) on the first day of treatment. Reinforcement with quinine on the first day only has not in our hands been followed by a similarly rapid alleviation of symptoms, and in any case the employment of this drug is undesirable in view of its association with the precipitation of blackwater fever. The mental disturbances associated with mepacrine therapy occur almost exclusively towards the end of a full course or during the first week after this has been completed, and it does not seem likely that they would supervene after a single day's reinforcement with this drug. Since paludrine in doses of mg. 300 twice daily for 10 days effectually sterilizes gametocytes for as long as these are present in sufficient numbers in the peripheral blood to infect mosquitoes, such a course, reinforced with mepacrine in massive dosage on the first day seems likely to fulfil the main objectives of treatment, namely rapid termination of the clinical attack, freedom from relapse, sterilization of gametocytes and minimum risk of

It is possible that reinforcement with mepacrine mg. 800 would prove sufficient for this purpose.

injurious side effects. We would advise, however, that this course be followed up by a maintenance dose of paludrine mg 100 daily for the ensuing 6 weeks, in order to cover the period during which falciparum relapses are most likely to occur. In endemic areas where paludrine prophylaxis is in force, the patient would come under this regime automatically on completion of the treatment given for the clinical attack. There are, however, many cases on record where individuals have experienced their first overt attack of malaria after leaving the endemic area, and such instances are likely to become more frequent in future with the progressive development of more rapid methods of transport. It is for the benefit of these cases in particular that specific instructions for the after treatment of malaria are most urgently needed.

SUMMARY

1 An account is given of a series of therapeutic trials with paludrine carried out at Horton Hospital, Epsom, against infections with a strain of *P. falciparum* obtained from an African child resident in Lagos, Nigeria, West Africa.

2 Paludrine effectually controlled the clinical attack produced by infections with this strain of parasite, but its action in this respect and in clearance of asexual parasites from the peripheral blood was somewhat less rapid than that achieved with mepacrine or quinine.

3 Paludrine without reinforcement failed to effect radical cure of infections with this strain, nine out of ten cases so treated relapsing within 3 weeks after completion of the course. This finding was in marked contrast with the results reported by FAIRLEY and his colleagues in their researches on infections with New Guinea strains of *P. falciparum*.

4 Radical cure of infections with the Lagos strain of *P. falciparum* was effected in a limited series of cases treated with paludrine reinforced with mepacrine or with quinine on the first day of treatment, and with quinine alone.

5 Following a course of paludrine mg 300 twice daily for 10 days, gametocytes were found to be non-infective to mosquitoes for as long as they continued to be present in the peripheral blood in sufficient numbers for infection to occur.

6 Reinforcement of a 10 days' course of paludrine with mepacrine given on the first day of treatment shortened the average duration of pyrexia and clinical symptoms by approximately 24 hours. Similar reinforcement with quinine did not have this effect.

7 It is considered that a course of paludrine mg 300 twice daily for 10 days, reinforced with mepacrine mg 900 given in three doses on the first day of treatment and followed by a maintenance dose of paludrine mg 100 daily for the ensuing 6 weeks would fulfil the main objectives in the treatment of

falciparum malaria, namely rapid termination of the clinical attack, a high radical cure rate, sterilization of gametocytes and minimum risk of injurious side effects.

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Dr. A. A. El-Dokki, M.D., F.R.C.S., F.R.C.P.,
 Dr. A. A. El-Dokki, M.D., F.R.C.S., F.R.C.P.,
 Dr. A. A. El-Dokki, M.D., F.R.C.S., F.R.C.P.

CHRONIC PULMONARY SCHISTOSOMIASIS A CLINICAL AND RADIOLOGICAL STUDY

by

M. EL-DOKKI

H. EL-DOKKI

A. M. MORSA

and

A. A. DUKKI

From the Department of Thoracic Diseases, Faculty of Medicine, Cairo University, Cairo, Egypt

Pulmonary schistosomiasis occurs in the early stage of schistosomiasis and in the late chronic stage. This clinical and radiological study was made on a series of 49 cases of chronic pulmonary schistosomiasis selected from 103 cases of urinary and intestinal schistosomiasis.

CLINICAL STUDY by M. EL-DOKKI and A. H. MORSA

Forty-nine cases of chronic pulmonary schistosomiasis were selected for clinical study. The diagnosis of the disease was made on clinical and radiological ground.

Age—The age varied between 10 and 55 years, the highest incidence being between 10 and 30 years. Cases below 10 years of age were not included in this series, as this age is not limited to Fayed Hospital, where this study was made.

Sex—Forty-four cases were males and five females. This does not represent the true sex incidence as in Egypt female patients do not readily enter hospital.

Occupation—Forty-three of the 49 cases were peasants working in the fields.

Onset—The onset was gradual. Two patients gave a history of fever preceding their illness.

Symptoms.—The following symptoms were complained of: dyspnoea on exertion, general weakness, cough, giddiness and fainting, palpitation, thoracic pain, praecordial pain and haemoptysis. Symptoms of urinary or intestinal schistosomiasis were common.

Dyspnoea on exertion was the most common symptom and was present even in the absence of anaemia. In two cases the dyspnoea was due to congestive heart failure. Cough was generally dry, sometimes accompanied by expectoration of mucus or mucopurulent sputum. Praecordial pain, dull aching in character, was sometimes complained of. Slight haemoptysis occurred in one case.

Physical Signs

Cyanosis of a mild degree was present in only three cases, and slight clubbing of the fingers in seven. Swelling of the superficial cervical veins was noted in two cases with congestive heart failure.

Heart.—The apex beat was usually in the normal position, in some cases in or slightly outside the left mid-clavicular line in the fifth or sixth intercostal space. Twenty-eight of the 49 cases had clinical signs of pulmonary hypertension and dilatation, viz. pulsation, diastolic shock, impairment of resonance in the second or third left intercostal space. Accentuation or splitting of the second pulmonary sound and a harsh systolic murmur were heard over the pulmonary area in the majority of cases, and a diastolic murmur over the same area in one case. A soft systolic murmur was heard over the praecordium in moderately or severely anaemic patients. No thrills were noted in this series.

The pulse rate varied from 70 to 110. Extrasystoles occurred in one case. The blood pressure varied from 90 to 133 systolic, and 55 to 90 diastolic.

Lungs.—In 34 out of the 49 cases no physical signs were found in the lungs. In 15 cases a few rhonchi or crepitations were heard over the bases of the lungs. Sputum could be obtained from seven cases; no schistosome ova were found in it, only eosinophils.

Liver.—The liver was enlarged in 41 cases; the enlargement was due to hepatic schistosomiasis. In the two cases with congestive heart failure the liver was enlarged in one and shrunken in the other from advanced cirrhosis of the liver.

Spleen.—The spleen was enlarged in 38 cases; in two it had previously been removed. The splenomegaly was due to hepatic schistosomiasis.

Ascites was present in ten cases and oedema of the lower limbs in five. The ascites was associated with advanced schistosomal cirrhosis of the liver.

Pyrexia was absent in all the patients except one who had acute pyelitis.

Blood Picture.—The haemoglobin varied between 15 and 104 per cent; the low figures were due to associated ancylostomiasis which occurred in six cases. The red blood corpuscles varied between 1,670,000 and 5,350,000 per c.mm.; the leucocytes between 2,400 and 16,200 per c.mm., and the eosinophil between 1 and 40 per cent.

Figueroa (1940) states that marked cyanosis in Ayerza's disease without failure of the right ventricle "indicates the presence of changes in either the capillaries or the pulmonary parenchyma interfering with gas exchange."

In our series polycythaemia was absent and clubbing of the fingers slight and uncommon. Failure of the right ventricle was late in its appearance as its cause is mechanical rather than myocardial. It was observed in two of the 49 cases. Slight haemoptysis occurred in one case due probably to pulmonary hypertension or pulmonary infarction.

In the diagnosis of cardiovascular schistosomiasis, causes of chronic cor pulmonale other than schistosomiasis have to be excluded, notably mitral stenosis, atrial septal defect, patent ductus arteriosus, pulmonary fibrosis and pulmonary emphysema. Mitral stenosis is differentiated by its characteristic apical signs and dilatation of the left auricle radiologically; atrial septal defect by early dilatation of the right auricle and hilar pulsation, patent ductus arteriosus by its characteristic murmur and high pulse pressure and pulmonary fibrosis and emphysema by their physical signs. It may be noted that even advanced pulmonary fibrosis or emphysema does not lead to the enormous dilatation of the pulmonary artery and conus which may occur in primary pulmonary endarteritis schistosomal or otherwise.

Antimony Treatment

This was given to 44 cases for the urinary or intestinal, as well as the pulmonary schistosomiasis. As mentioned previously four cases of chronic bronchitis and one case of bronchial asthma improved on this treatment. Fever, urticaria, acute pulmonary congestion and marked eosinophilia were observed in some cases. One patient died suddenly: a postmortem could not be done.

The pulmonary reactions to antimony treatment have been attributed to allergy or to focal reaction round the lung lesions: these have been observed, radiologically to enlarge and then regress. Their clinical manifestations are bronchial asthma and focal pneumonia. The latter may be verminous, due to embolization of schistosome worms from the veins of the urinary or intestinal tracts.

Antimony compounds have to be used with caution in pulmonary schistosomiasis, especially the cardiovascular form. The right ventricle, already strained by the pulmonary hypertension, will stand poorly their direct toxic effect on the myocardium and the general and acute pulmonary reactions which may follow their administration. They are definitely contraindicated when the right ventricle has failed.

RADIOLOGICAL STUDY BY H ERFAN AND A A DEEB

Forty-nine cases of chronic pulmonary schistosomiasis were selected for radiological study. They are the same cases selected for the clinical study.

The aim of this radiological study is the establishment of a definite radiological picture for chronic pulmonary schistosomiasis. Few radiological observations have been published of which MAINZER's paper (1938) is probably the most important.

Technique

A systematic radiological examination of the chests of 103 cases of urinary or intestinal schistosomiasis was made. Radiograms of the chest were taken showing postero-anterior and oblique views, and in addition the chest was screened with the aid of a "barium swallow," to exclude dilatation of the left auricle. In some cases bronchography was done. A definite radiological picture could be obtained in 49 out of the 103 cases.

Classification

The radiological pictures of the 49 cases were classified into three grades as follows:

Grade I Focal arterial changes

Grade II Widespread arterial changes with slight heart changes

Grade III Widespread arterial changes with gross heart changes

GRADE I Focal arterial changes In this grade there is intensification of the shadows of one or more of the second or third degree arteries, mostly the basal. These arteries have the following characteristics: (a) Wider diameter than normal, (b) markedly denser shadow, (c) moderately tortuous course and sometimes irregular contour, so that they appear beaded (rosary arteries), (d) irregular and hazy outline.

The affected lung fields show a conspicuous and more intricate network of arterioles as compared to the few main blood vessels normally seen. In many cases branches other than the basal may present the same appearance.

The hilar regions, which are moderately enlarged, show uniform rounded or elliptical mottlings of varying diameter and density. These mottlings are sclerotic and dilated arteries end on. The nodules which give the arteries their beaded appearance are seen overlapping the shadows of the small arteries or in their immediate vicinity. They are small, about 0.5 to 1 mm in diameter, not very regular in shape and more or less hazy in outline. The presence of these nodules is the criterion of the activity and intensity of the infection.

GRADE II This is of two types: (a) Active type, (b) healed type.

(a) *Active Type*—In this type the lesions are more widely distributed than in the previous grade. Throughout the lung fields, specially the bases, the intensification of the arterial shadows is striking, with marked tortuosity and beading. There are clusters of mottlings in relation to the arteries, giving the lung fields a granular background.

The hilar shadows are bigger denser and show the typical mottlings previously described. Their outer border is more or less irregular the irregularity being due to the dense shadows of the arteries proceeding distally from the hilum.

The subaortic notch is either partially obliterated in its upper portion when the pulmonary trunk is dilated or abolished with a slight bulge when conus and trunk are both dilated. This prominence partly masks the shadow of the left main branch which appears less enlarged than the right, especially if we take into consideration its normal direction backwards. The left branch, however, is as much dilated as the right and can be better seen in the left oblique or semi-oblique view.

The cardiac shadow is moderately enlarged in the transverse diameter and more or less globular in form (hypertrophic), with a mitralized configuration. The left ventricle is not enlarged, the right ventricle is enlarged and the left auricle is never enlarged. The aorta is normal, sometimes hypoplastic, and the aortic knob is seen, except when it is partly masked by the dilated conus and trunk of the pulmonary artery. On screening pulsations of the hila are normal or less than normal.

(b) *Healed Type*—In this type the arteries and arterioles show the same changes as in the active type, but there are fewer nodules with only moderately granular lung fields. The same cardiac and hilar changes are present as in the active type.

GRADE III This is also of two types (a) Active type (b) healed type.

(a) *Active Type*.—This is met with in the old standing neglected cases with repeated infections.

The lesions are widespread in both lungs but are more marked in the bases and medial zones.

Apart from the intensification of the pulmonary arterial shadows with their tortuous and beaded appearance, the perivascular mottlings are so dense and widespread that patches of localized opacities may appear here and there due to the aggregation of these mottlings. The combination of these shadows with different components formed by blood vessels, perivascular nodules and small translucent areas give the picture of pseudo-honeycombing. The use of a magnifying glass is very helpful in identifying the various components of this picture, especially if we follow the course of different arteries. The pulmonary conus and trunk of the pulmonary artery are ballooned, may reach aneurysmal size and mask the left pulmonary branch and the shadow of the aorta and its knob. Aneurysmal dilatation of the primary pulmonary branches is also seen with dense shadows which may be due to organized clots.

The heart is increased in transverse diameter with the typical configuration of cor pulmonale. The right ventricle and right auricle are enlarged but the left



FIG 1—Radiograph of Grade I type Note the beaded and tortuous arterial shadows



FIG 2—Radiograph of Grade II active type Note the granular lung fields, beaded arteries, increase of the hilar shadows and prominence of the pulmonary conus



FIG. 3.—Radiograph of Grade II healed type. Note the moderately granular lung fields, increase of the hilar shadow, and prominence of the pulmonary cross.



FIG 4—Radiograph of Grade III active type. Note the granular lung fields, increase of the hilar shadows and marked prominence of the pulmonary conus.



FIG 5—Radiograph of Grade III healed type. Note the moderately granular lung fields, increase of the hilar shadows and marked decrease of the pulmonary conus.

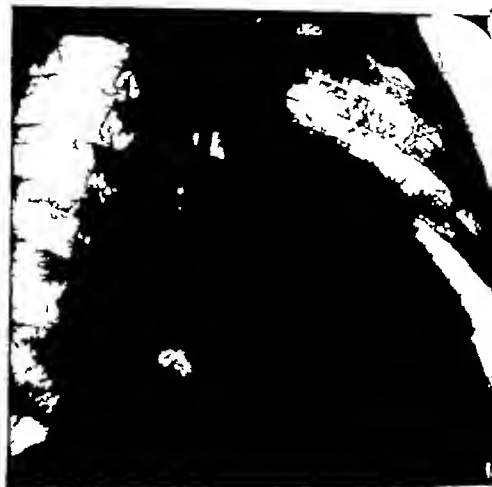


FIG. 6.—Radiograph of Grade III active type right oblique view. Note the enlargement of the Beck pulmonary marked prominence of the pulmonary cones and the non-dilatation of the left atricle.

auricle is never enlarged The left ventricle may be moderately enlarged The aorta is usually normal or hypoplastic The shadow of the superior vena cava is occasionally prominent

(b) *Healed Type*—In this type the same arterial and heart changes are seen but there are few nodules with more or less clear lung fields In some cases of the active types, in Grades II and III, small localized patches of homogeneous opacity in the lung fields were observed These may be infarcts or areas of focal pneumonia due to schistosomes Calcifications in the lung fields were sometimes seen They may be tuberculous as the hilar glands were often calcified In cases with ascites the picture of the bases is masked by the high diaphragm and consequent distorted heart configuration The hilar shadows and the heart, however, show the same characteristic changes but the upper lung fields are usually free

Differential Diagnosis

The following are the main diseases which may resemble pulmonary schistosomiasis radiologically

(1) Miliary tuberculosis The nodules of miliary or submiliary tuberculosis have a generalized distribution and are more marked in the upper lobes Enlargement of the hilum, if present, is glandular and not vascular The course of the arteries in the lungs is masked There are no heart changes

(2) Periarthritis nodosa In this condition the hilar shadows are enlarged with mottled appearance in the lower and middle lung fields This is more marked towards the central region, thus conforming with the distribution of the large and middle-sized arteries The periphery is always spared Sometimes there are transitory infiltrations of widespread haziness in the middle zones, but again sparing the periphery

(3) Loeffler's syndrome In this condition there are lesions 2 to 5 mm in diameter with a dense centre and blurred edges Mottled areas are more numerous and larger in the hilar regions The lesions are parenchymatous and not perivascular

(4) Passive hyperaemia, as in heart failure or conditions causing venous obstruction The vascular tree becomes ill-defined in the early stages In advanced cases there is marked loss of translucency and blurring of the lung fields

(5) Active hyperaemia, as in atypical pneumonia. The main vessels are dilated and there is no beading or tortuosity of the arteries There is also loss of translucency of the lung fields

(6) Interatrial septal defect and other congenital heart conditions There is early enlargement of the right auricle, hilar pulsations and absence of arterial beading and perivascular nodulations

Tartar emetic reactions The radiological appearances in the cases which show the reaction, whether the course of tartar emetic is prolonged or intensive

are similar in their main features. In the intensive course, however the reactions occur earlier and are more marked. The cardiac shadow is increased in transverse diameter sometimes, by as much as 1 cm. The hilar shadows become larger and denser with a hazy outline. The lungs are studded with small or big patches of opacity which may be more marked in one lung than in the other and are mostly basal or paramedian in distribution. These patches are dense in the centre and give a hazy outline and resemble very much those produced by bronchopneumonia or active congestion of the lungs. They are sometimes seen to develop round lesions in the lung present before treatment. Pleural reaction in the form of a slight pleural effusion may occur.

These reactions to tartar emetic gradually subside the lungs become clearer and the heart regains its previous size.

SUMMARY

Forty nine cases of chronic pulmonary schistosomiasis were selected from 103 cases of urinary or intestinal schistosomiasis, for clinical and radiological studies.

The symptoms complained of were dyspnoea on exertion, weakness, cough, giddiness and fainting, palpitation, thoracic pain, praecordial pain and haemoptysis.

The heart showed, in the majority of the cases, a normal position of the apex beat and the signs of pulmonary hypertension and dilatation.

The lungs showed no physical signs in most of the cases, and a few rhones and crepitations at the bases in some.

The sputum contained no schistosome ova, only eosinophils.

Cases of chronic bronchitis, bronchial asthma, pulmonary emphysema and pulmonary fibrosis were observed. Four cases of chronic bronchitis and one case of bronchial asthma improved on antimony treatment.

Cases of the cardiovascular type with the symptoms and physical signs of primary pulmonary endarteritis were more common. Cyanosis was slight and uncommon. Polycythæmia was absent. Congestive heart failure occurred in two cases.

Antimony treatment was given in most of the cases. Improvement followed in some and reactions in others. The reactions were fever, urticaria and acute pulmonary congestion.

Radiologically three grades are described.

In Grade I there are focal arterial changes in the lungs. The small arteries, especially the basal, become wider in diameter than normal, with a dense shadow, tortuous course, irregular hazy outline and beaded appearance. The affected lung fields show a conspicuous and more intricate network of arterioles as compared to the few main blood vessels normally seen. The beaded appearance is due to nodules, about 0.5 to 1 mm. in diameter overlapping the shadows of the small arteries or lying in their immediate vicinity.

In Grade II there are widespread arterial lesions and slight heart changes. The arterial lesions in the lungs are more widespread than in the previous grade. The hilar shadows are bigger and denser than normal. The subaortic notch is partially or entirely obliterated. The cardiac shadow is moderately enlarged in the transverse diameter and has a mitralized configuration. The right ventricle is enlarged, the left ventricle not enlarged, and the left auricle never enlarged. The aorta is normal and the aortic knob is seen, except when it is partially masked by the dilated pulmonary conus and trunk of the pulmonary artery.

In Grade III there are widespread arterial changes and gross heart changes. The arterial lesions in the lungs are similar to those in the previous grade. The pulmonary conus and trunk of the pulmonary artery are ballooned, may reach aneurysmal size and mask the shadow of the aorta and its knob. Aneurysmal dilatation of the primary pulmonary branches is also seen. The heart is increased in the transverse diameter with the typical configuration of cor pulmonale. The right ventricle and right auricle are enlarged, the left auricle is never enlarged and the left ventricle may be moderately enlarged. Tartar emetic reactions observed radiologically, sometimes occur. The cardiac shadow is increased in the transverse diameter. The lungs show small or big patches of opacity resembling those of bronchopneumonia. These sometimes develop round lesions in the lungs seen before treatment. A slight pleural effusion may occur. The reactions to tartar emetic gradually disappear.

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DIETARY DEFICIENCIES IN CHILDREN IN THE ISLAND OF VITI LEVU, FIJI

BY

F ADAM THOMSON, MRCS, LRCP.*

Medical Officer, Fiji

A clinical survey of 7,281 school children and of over 200 pre-school children in the Island of Viti Levu, Fiji, in the Pacific, was undertaken in 1947. The following observations on the nutritional state of these children are of interest as an indication that nutritional deficiencies in Fiji, although present, are not of such severe degree as those reported by PLATT and other observers elsewhere in the Colonial Empire. They are also of interest as the picture is uncomplicated in Fiji by the presence of malaria or other blood-destroying tropical diseases. Hookworm infestation is common (more than half the children examined were infected) but, as other observers have noted, this does not seem to bear a direct relationship to the degree of anaemia present nor to the general state of health.

The economic state of the people is better than in many parts of the world and serious poverty is almost unknown. The staple crops, rice for the Indian, dalo (taro) and cassava (tapioca) for the Fijian, can be grown with reasonable ease, and although there is, as elsewhere, some post-war shortage of these there is no real starvation.

The children in the survey were all in urban areas or in villages where store goods were available. Urbanization is spreading, and there is among both Fijians and Indians a drifting away from the traditional ways of life and diet. Native foods are falling into disuse and store goods are preferred to the fruits of the people's own labours. Bread, sweetened tea taken without milk, and tinned beef are becoming more and more the people's taste. Indeed, white bread, tea and sugar are becoming the staple diet of most of the smaller children. The liking for expensive European foods is increasing at a greater rate than is the family budget, and such European foods as are in use are not used to the best advantage owing to ignorance of and lack of interest in suitable methods of cooking.

The Indian family has, as a rule, a more varied diet than does the Fijian, but here other factors are at work as the increase in the number of the family is commonly more rapid than is that of the household earnings. Such traditional foods as are in use are not utilized to the best advantage. Rice is over-milled, meat and green vegetables are over-cooked and fish and coconuts are

*By kind permission of J. C. R. BUCHANAN, M.D., F.R.C.P., D.T.M. & H., Inspector-General South Pacific Health Service.

not used to the extent that in the past was the rule. To make matters worse, Fijian custom demands, and this custom still largely persists, that the adults in the family shall eat before the children.

Some attempt was made to work out the food values of the different diets, and there is no doubt that there is unbalance in the ingredients. Calorie values as a rule are adequate, though heavily on the carbohydrate side and there is a shortage of minerals and of vitamins. That this is so was confirmed by Miss ABRAHAM, B.L.Sc. who in a 3½ months survey in 1947 reviewed the records of island dietary custom and the composition of local foodstuffs for the South Pacific Board of Health. In her opinion, although the energy value of the typical family diet is adequate, it is unbalanced owing to carbohydrate excess and is largely made up of white bread and sugar. That this should be so is particularly regrettable as the native carbohydrate staples can be grown with reasonable ease, fish is available or could be made so and coconuts and citrus and other fruits grow easily.

Defects that have come to be associated with a badly balanced diet occur frequently. Teeth are bad, mouths are unhealthy and there are changes in skin texture and in muscle tone, indicating that diets are both deficient and unbalanced. These defects are not as a rule of severe degree and seem to indicate a sub-optimal nutritional state rather than a condition of gross malnutrition.

As is the rule, the child first shows signs that something is amiss soon after the weaning period. These signs are seen as a defect of the dental enamel as early as the eleventh month and by 5 years of age many other signs of nutritional ill health are present. It is in the 5 to 8 age group that the highest proportion of the children show these defects. 65 per cent. of all the children examined showed one or more signs attributable to malnutrition.

The systematic examination of the children was carried out at the different schools in town and village. The following was the race distribution: Indians 3,214; Fijians, 2,530; part Europeans, 710; Europeans, 340; Chinese 147 mixed, 345—a total of 7,281.

The age range of school children was from 5 to 18 years: the pre-school children from birth to 5 years.

For the purposes of this paper only figures relating to Indian and Fijian are quoted in full because it is thought that factors other than diet influence the figures relating to other racial groups. It may be mentioned, however that similar signs of deficiency were found in all racial groups though frequency and intensity of incidence were steadier in the two main groups.

The usual signs of nutritional ill health were looked for and recorded, and the following notes show the standards used. The relevant figures are summarized in a table at the end.

Height and Weight

All children were weighed and measured and the results only confirmed that height and weight are very little guide as to the state of nutrition. This is



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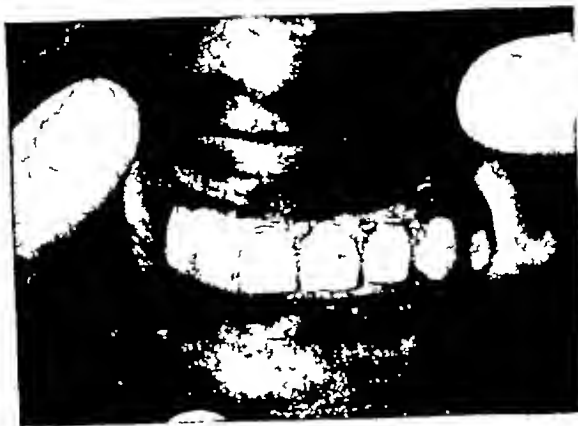


3

- (1) 18 months Fijian female (Tiranga) Black discoloration and roughening of surface of temporary teeth at gum margins Gums swollen and congested
- (2) 4½-year old Fijian male (Vereti) Temporary teeth show destruction of enamel and discoloration Gums swollen and congested
- (3) 7 year old Fijian female (Unaia) Destruction of temporary teeth Permanent incisor erupting Gums swollen and congested



- (4) 11 year-old Fajan male (Epilepsy). Permanent teeth show commencing wear as enamel at gum margin. Gums swollen and congested.
- (5a) 12 year-old Fajan male (Trisomy 21). Discoloration and spaces in enamel at gum margins. Gums show swelling and congestion.
- (5b) 12 year-old Fajan male (Trisomy 21). Advanced destruction of 6-year-old molar.



6



7

- (6) 13 year-old Fijian female (Salome) Defect of enamel with roughening and pitting
Gums swollen and congested
- (7) 15 year-old Fijian male (Pita) Advanced caries of permanent incisors, also discoloration of pre molars Gums show some swelling and congestion

NOTES

The defects shown are all frequently found (ranging from 33 per cent defective in 1 065 children at boarding schools to 87.7 per cent defective in 180 village children) and seem definitely, to be related to the changing diet of the Fijian. Adult Fijians, on the whole, do not show the same defects in the permanent teeth that are shown in this series, and it is true that during the past ten to fifteen years the diet has considerably changed.

The type of defect shown in the photograph of the 18 months child in this series commences soon after the child is weaned, and changes in the teeth are visible at eleven months old or soon after.

The change is sudden from breast milk to a diet of dry bread and sweetened tea without milk. A lump of cassava is also given to the child to chew. As the child grows older the diet improves but little, and is still grossly short of calcium, vitamins A, B and C and protein.

It is noteworthy that Gilbertese children who have spent their early years in the Gilbert group have sound teeth. (In a series of 49 children only one child showed defective teeth). The staple diet of these children is coconuts and fish.



60 old Indian female (V jay). Rash of many years duration. Rough, well
pigmented. 15.11.47. Treated seven days with vitamin and zinc. 60 re-obsd
11.4. Skin less rough and less pigmented.

P ADAM THOMSON

particularly noticeable in Fiji, where there are so many racial types The A C H index was not used

Muscular Tone

Muscular tone was frequently found to be poor and associated with faulty posture Lordosis and postural scoliosis were not uncommon Poor muscle tone was only recorded as a sign when muscles were definitely flabby and posture bad Due allowance was made for racial differences in physique The Knudsen Schiotz sign of the median dorsal furrow frequently occurred

Teeth

Defective teeth were recorded as such when one or more of the following occurred —

- (a) Caries of more than one tooth of the temporary dentition
- (b) Caries of one or more teeth of the permanent dentition
- (c) Mottling of two or more teeth
- (d) Crowding and/or badly formed jaw and palate
- (e) Fillings and/or extractions

mouth

(a) *Oral Sepsis*—Varying types of unhealthy mouths were found—septic conditions, gingivitis, overgrowth of mucous membrane, swollen, spongy and bleeding gums Many mouths showed a combination of these conditions

(b) *Eroded Tongue*—There were a few cases showing slight degrees of fissuring and erosion of the mucous membrane of the tongue No severe cases were found

(c) *Angular Stomatitis*—Superficial erosion or slight maceration and fissuring of the mucous membrane of the lips at the angle of the mouth was occasionally found

(d) *Per Lèche*—A few children showed dryness and marked vertical fissuring of the lips

Eyes

Most children showed some degree of thickening and pigmentation of the conjunctivae over the outer quadrant of the sclerotics A few showed typical Bitots spots Photophobia and lachrymation and complaints of poor vision—borne out by a visual test—were often found

Skin

(a) *Dry Skin*—This was recorded when there was loss of the normal gloss of the skin associated with varying degrees of dryness and loss of elasticity This condition seemed to merge in the more severe cases into the true mosaic condition

(b) *Dry Skin with Mosaic*—All cases of dry skin with mosaic, showing severe degrees of dryness and fissuring of the epidermis with a wide distribution, were recorded in this group Typically present on shins and thighs, in many children the eruption was widespread The moist varieties described by WILLIAMS in her description of crazy pavement eruption were not seen in this

series, nor were the depigmentation, oedema and hair changes described by her seen at all.

(c) *Phrynodermia*.—Varying degrees of this condition of dryness and papular eruption were found. It was typically found on the extensor aspects of arm and forearm, but in some cases was much more widespread and gave the rough "nutmeg grater" feel over large areas of limbs and trunk. In these cases papules could be clearly seen and felt and were as a rule pigmented. In some cases the condition was associated as well with the mosaic type of eruption.

(d) *General*.—To summarize children were found with the following types of skin lesions —

(i) *Phrynodermia* alone with wide distribution or in some cases limited to localized areas on the extensor aspect of arms and forearms.

(ii) Mosaic eruption, usually widespread but sometimes localized to the typical shins and thighs.

(iii) A combination of phrynodermia and mosaic—a papular eruption and as well rough, irregular scaldness the mosaic pattern being generalized over the body.

(iv) A general roughness and scaldness over the body associated with patchy pigmentation. Pigmentation in some cases had butterfly-patch distribution on the face and on extensor aspects of hands and feet suggesting that the eruption was pellagrous in nature. This latter type of eruption responded to nicotinic acid given in doses of mg. 100 per day for 10 days to two children of nine years. With this treatment dryness scaldness and pigmentation disappeared to great extent.

Infections.

Infections of different types were common. Unhealed sores were frequently found, a history of diarrhoeas and respiratory tract infections frequently obtained and profuse and persistent nasal catarrh often observed.

Anaemia.

Anaemia was not found to be frequent or severe. Among 263 children only four were found by the Tallqvist method to have haemoglobin as low as 70 per cent. None was below this figure. Pallor of mucous membranes suggesting some degree of anaemia was found in less than 1 per cent of all children examined.

Hair Changes.

Hair changes were not observed. Most children, however, treat their hair with coconut oil and changes may have been masked by this.

Bradycardia.

Pulse rates were checked at two schools only. Thirty four per cent. of Fijians gave pulse rates lower than the accepted standards for their ages. 18 per cent. of Indians also showed low pulse rates.

Pigmentation of Tongue Papillae

Tongue papillae were found to be pigmented in many cases which showed skin changes. The degree varied from pigmentation of a few to nearly all papillae showing changes.

VITI LEVU, FIJI	
SIGNS OF NUTRITIONAL ILL HEALTH IN SCHOOL CHILDREN	
	with defects

SIGN OF NUTRITIONAL ILL HEALTH IN SCHOOL CHILDREN																	
Race	Number of children	Poor muscle tone and faulty posture		Teeth defects		Mouth defects				Skin							
						Oral sepsis		Sore tongue	Cheliosis	Dry skin		Phrynoderma		Mosaic			
		No	Per cent	No	Per cent	No	Per cent	No		No	Per cent	No	Per cent	No	Per cent.		

DENTAL AND NUTRITIONAL DEFECTS

DENTAL AND NUTRITIONAL DEFECTS							
						5-8 years	
						Dental defects	Percentage defective
						Nutritional defects	Percentage defective
Indian	3,214	341	10.6	1,000			
5-18 years							
Rice							
Fujian							
Indian							

CONCLUSION

It is suggested that all the signs which have been described point to a degree of imbalance in the diets of the people of Fiji and that although there is no real starvation there is a shortage, particularly of protein, vitamins A, the B complex and C, and possibly of calcium.

Although this paper is intended mainly as a record of clinical findings, it may not be out of place to mention that one or two observations definitely showed that such imbalance of diet as is present is not of severe degree and is easily correctible.

During the year at periodic visits to schools, it was noticed that there is a definite seasonal variation in the severity of some of the signs. During the fruit season bleeding gums were definitely less frequent and less severe and with 8 months cod liver oil (4 000 international units vitamin A daily) skin changes, especially prurigo, and muscle tone showed considerable improvement. The fact that as the child grows older there is, as a rule, some improvement in health also suggests that dietary faults, grave in the early years, become less grave as the child grows towards adult life. The older child able to follow more adult pursuits may obtain fish and wild fruits, and is also able to eat larger quantities of the staple articles and so by bulk make up for lack of variety.

The serious aspect of the deficiencies which have been mentioned is, of course, the permanent nature of some of them and the increased incidence of serious infections associated with a lowered resistance to disease.

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THE TREATMENT OF POLYCYTHAEMIA VERA A RECORD OF ONE CASE TREATED WITH ANCYLOSTOMA INFECTION

BY

H F NAGATY, PH.D (CAIRO), M.Sc (LIV.),
Assistant Professor of Parasitology,

AND

A F ZANATY M.R.C.P (LOND), D.T.M. & D.P.H (CAIRO)
Assistant Professor of Tropical Medicine
From the Faculty of Medicine, Kasr-el-Aini, Cairo, Egypt

The treatment of polycythemia has passed through various eras with different drugs. No drug has been found to be curative, and results have shown much variation as recorded by different authors. The disease is due to increased activity of the bone marrow with special selective activity of the erythron. Some cases pass ultimately into myelosis.

The patient suffers from excess of blood with increased viscosity and concentration of red blood corpuscles. Various methods have been devised for treatment. *Ancylostoma* infection was tried by DUVOIR *et al* (1940) for the first time in 1940 on a woman and since then no further instance of this kind of treatment has been recorded. Our case is thus the second of polycythemia vera in which treatment by *Ancylostoma* infection is described. A discussion of the different methods of treatment and the reasons for choosing the *Ancylostoma* infection method is discussed later on in the text.

TABLE I.

Date	Examination	Infective lesions	Number of worms	Number of red cells per cmm.	Haematocrit per cent.	Colour index	Total leucocytes per cmm.	Eosinophils per cent.	Neutrophils per cent.	Segmented per cent.	Total polymorphonuclears per cent.	Lymphocytes per cent.	Monocytes per cent.
27.2.48	1st ex.			1,000,000	140		12,400			12	12	12	1
28.2.48										12	12	12	
30.4.48										12	12	12	
7.9.48										12	12	12	
14.5.48										12	12	12	
19.6.48										12	12	12	
4.7.48										12	12	12	
21.7.48				1,250,000	125	0.88	13,000			12	12	12	
22.8.48				9,400,000	118	0.85	16,800			12	12	12	
23.8.48				8,850,000	115	0.82	17,800			12	12	12	
29.10.48			22	6,800,000	125	0.8	17,000			12	12	12	
27.11.48										12	12	12	
17.12.48										12	12	12	
19.12.48	120 ex.									12	12	12	
12.2.47										12	12	12	
18.2.47										12	12	12	
20.2.47										12	12	12	
3.2.47				9,100,000	118	0.84	8,200			12	12	12	
14.6.47										12	12	12	
19.6.47										12	12	12	
20.6.47										12	12	12	
28.6.47										12	12	12	
28.6.47										12	12	12	
28.7.47				9,100,000	100	0.71				12	12	12	
13.9.47				8,100,000	85	0.62				12	12	12	
4.10.47				9,700,000	83	0.72				12	12	12	
9.11.47				8,180,000	48	0.63				12	12	12	
				5,000,000	68	0.8				12	12	12	

CASE REPORT

Z. S., the subject of this communication, who is a government clerk of about 55 years of age, is rather tall (181 cm) and thin (54 kgm). Formerly, i.e., 10 years ago, when his illness started, he weighed 74 kgm, but gradually lost weight. His main complaint was giddiness, especially when standing for some time, headache, incapacity for work or mental concentration, discomfort after meals, chronic dyspepsia and constipation. There was tightness of the chest exaggerated by walking, palpitation and sometimes he could not walk for more than 10 minutes. The patient was nervous and very short tempered. His past history showed no important illness except for an attack of malaria in 1921 which was treated successfully.

On examination the patient showed a congested face, congested conjunctiva, mouth and pharynx. The latter had the appearance of red velvet. This colour was noted by the patient as far back as the commencement of the other symptoms. The lungs and heart showed normal findings, the pulse-rate being 75 per minute and the blood pressure 110/70. The spleen was slightly enlarged and firm. The liver was also slightly enlarged with rounded edge. The patient sought medical advice for many years and had many different diagnoses and types of therapy including tonics, iron, etc. He was advised to have his teeth extracted, and as a result he has now a fitting of upper and lower denture.

He was sent for a blood test on 27.2.46. On taking blood from the cubital vein it was noted that it was very viscous and could hardly flow through the wide needle used. The blood picture (Table I) showed a marked increase of both haemoglobin and the number of the red blood corpuscles. The total leucocyte count was within normal limits and the differential picture showed only a shift to the left. Platelets were abundant in the film. The picture was therefore characteristic of polycythemia vera. No malaria parasites were found in film and thick drop preparations. The Wassermann and Kahn reactions were negative.

The patient was admitted to Kasr el Yami Hospital on 6.4.46, and was kept on normal diet. Mist rhei and soda was given three times daily. On examination his urine was found to be normal, his stools showed a few *Heterophyes heterophyes* ova but no *Ancylostoma* ova or *Entamoeba histolytica* cysts or vegetative forms. A sternal puncture was made on 14.4.46 and the report shows a mild hyperplasia with normal differential picture (Table II).

TABLE II

To il bone marrow		74 000 per c.mm.	Differential picture	
Ba ophiles	0.0 per cent.		Myceloestes	7.5 per cent.
Eos nophiles	2.0 "		Lymphocytes	11.0 "
Juveniles	3.5 "		Monocytes	0.5 "
Stall nucleated	17.0 "		Normoblasts	76.0 "
Segmented	11.0 "		Macrophils	10.0 "
Myceloblasts	0.0 "		Reticular cells	2.5 "
Promyelocytes	5.0 "			

After reviewing the different methods of treatment available, it was decided to produce a helminthic anemia by inducing infection with *Ancylostoma duodenale*.

On 20.4.46 resection of 120 c.c. of blood was made to give temporary relief to the patient. On the same day coproculture for *Ancylostoma* larvae was prepared by emulsifying small portions of faeces from an ancylostomiasis patient with little fresh water in the bottom of several petri-dishes. The covers of these dishes were lined with wet filter paper and the dishes were then left in a warm place and inspected and moistened daily.

Ten days after culturing, the patient was given 150 infective *Ancylostoma* larvae obtained by washing the filter paper lining the petri-dish covers while at sink with a few drops of fresh water, pipetting these off and applying them to the patient's hands and feet, waiting each time for the drops of water containing the larvae to dry completely.

The patient was discharged from hospital on 1.5.46. On 7.5.46 culture of 30 larvae was applied to the abdomen, and 6 days later similar quantity to the back. The drops of water containing the larvae were spread over as wide an area as possible so as to allow quick evaporation and penetration, and thus increase the chance of greater infection. This was an improvement on our first method, and on that of DUBOIS *et al.* of applying the larvae to the thighs as the areas finally chosen are more extensive and the infected water is less liable to roll off. After both applications the patient complained of dermatitis and on 4.6.46 he reported that he had also tracheitis and diarrhoea. These symptoms are due to migrating stages of *Ancylostoma* larvae before establishing themselves in the small intestine of the host, and prove the success of the infection.

On the 15.6.46, 200 c.c. of blood were drawn from the patient to relieve him of his discomfort.

On 4.7.46, the number of red cells and the haemoglobin level were less than at the previous examination although still higher than normal. The total leucocyte count was about 15,000. There was thus a moderate leucocytosis which was eosinophilic in nature—a characteristic feature of infection with intestinal helminths, with a shift to the left of the polymorpha. Platelets were abundant in the film. (Table I.)

On 31.7.46 the blood picture showed that the haemoglobin and red cells were still high but showed an improvement over the last examination. There was still a moderate leucocytosis which was eosinophilic in nature. Platelets were abundant in the film. (Table I.)

On 22.9.46 both the number of red cells and the haemoglobin level had risen slightly (Table I). Leucocytosis with a high eosinophilia was maintained. The patient reported that he had spent 1 month at the seaside at Nersa Malrouh and that he felt much better—the giddiness had disappeared and there was more aptitude for work than before.

An egg count by the Stoll method was performed on 23.9.46, from which it was deduced that the patient harboured only 32 worms.

On 29.10.46 there was a further slight rise in the number of red cells and the haemoglobin level remained at about the same figure but the total leucocytic count had fallen to 12,000. There was a slight fall in the eosinophilia and a rise in the number of polymorpha. (Table I.) Owing to the small number of ancylostomes the patient was found to harbour and to the rising red cell count, it was decided to give him another infection and for that reason a fresh culture was made and on 27.11.46 about 150 infective larvae were applied to his back and on 17.12.46 a similar number to his abdomen.

On 19 12 46, 120 c.c. of blood were drawn out for relief. On 12 2 47, the patient sent a sample of his stools for egg count, and stated that he felt much better than he ever did before. An egg count was made and it was deduced that the patient harboured 87 worms.

The blood picture on 18 2 47 showed a reduction in the red cell count and a fall in the haemoglobin level. The leucocyte count had also fallen and the eosinophilia was less pronounced. The number of polymorphs continued to rise (Table I).

A further 250 infective *Ancylostoma* larvae were applied to the back and to the abdomen, at a week's interval, each prepared from a fresh faecal culture. On 14 4 47, a Stoll egg count showed that the patient might be assumed to harbour 420 worms. A similar count was made 2 days later, which yielded a figure of 344 worms present, while on 20 4 47 the count was 240 worms.

On 29 4 47 the number of red cells remained as before, but the haemoglobin level had fallen to 100 per cent for the first time, while the eosinophilia was also lower (Table I). On 8 6 47 the red cell count had fallen to a normal figure for the first time while the haemoglobin level had dropped to 85 per cent. Polymorphs had risen to 60 per cent, while eosinophils had fallen to 23 per cent (Table I). On 28 7 47 a slight rise in the red cell count was observed but the haemoglobin level remained at 85 per cent. On 4 10 47 the red cell count was once more normal, while a further fall in the haemoglobin level to 65 per cent was observed. There was also a slight rise in the eosinophilia while the total white cell count had increased to 15,400 (Table I).

On the date of the last examination, 5 11 47, the red cell count was normal and the haemoglobin level had fallen still further to 60 per cent, the total leucocyte count was still high with pronounced eosinophilia, but no other significant abnormality (Table I).

The blood count was, then, almost normal.

It will have been noted that the number of larvae administered was much higher than the number of adult worms harboured by the patient, which may be attributed to two factors, namely, that the larvae do not all penetrate and that not all of those which do penetrate succeed in reaching the alimentary tract.

SUMMARY OF THE BLOOD CHANGES

The red cell count in this case rose to a peak value of 8 million, in similar cases the count ranges from 7 to 12 million per c.mm.

The haemoglobin level was also correspondingly higher than normal at its maximum (140 per cent). The colour index, however, remained always less than unity.

The leucocyte count fluctuated between 8,000 and 17,000 per c.mm. In other cases this count averages about 20,000 and may sometimes reach extremely high levels (50,000 to 114,000).

The differential count in this case shows a shift to the left of the polymorphonuclears. During the acute *Ancylostoma* infection there was a leucocytosis with high eosinophilia. Generally speaking *Ancylostoma* infection does not produce such a high eosinophilia as in *Schistosoma* infection, the former being an intestinal infection whereas the latter is a systemic infection.

I chronic myelostomiasis a mild degree of eosinophilia occurs (3 to 10 per cent as seen by us in routine examination in the wards. In acute infection as in the case during iron treatment of chronic cases there is usually a leucocytosis with an eosinoph. which can reach high level

DISCUSSION

Treatment of this malady is based on an attempt to reduce the number of red blood corpuscles. The disease is attributed to many aetiological factors, the most recognized of which is a hyperplasia of the haematopoietic tissue of the bone marrow indicated by the large number of the red cells, the presence of immature forms (reticulocytosis), the leucocytosis with immature forms present at the same time and the enlarged spleen. This hyperplasia is more selective to the erythron and in rare cases myelosis may be superadded.

Another possible causative factor of the excessive erythropoiesis is the abundant production in the stomach of the haematopoietic principle "adamin." With these causes in mind, treatment is generally carried out by one or more of the following methods

(1) *Lee's method*.—This was done several times in our case for quick relief and only small quantities of blood were taken. For proper treatment by this method large quantities, half to one litre are removed and this repeated at 8 to 10 weeks interval. An iron-poor diet is prescribed at the same time. This method is perhaps the safest of all, but the effect is transient and the procedure is troublesome on account of the high viscosity of the blood.

(2) The administration of drugs, phenyl-hydrazine and the less toxic acetylphenyl-hydrazine which have well-marked haemolytic action, may be employed. The most dangerous draw-backs of these drugs are the production of thrombosis or very rapid haemolysis of the red cells while leukaemic changes which may prove fatal can occur. For these reasons we did not attempt to use this type of treatment, which is moreover unsuitable for weak, bed-ridden patients or those with arterio-sclerosis or previous history of thrombosis. The drugs have a cumulative effect which continues after the withdrawal.

(3) Radiation therapy. This, which is said to give good results is done with a view to reducing the hyperplasia of the bone marrow. An excessive dose will cause aplastic anaemia while small doses will have stimulating effect. The dose therefore must be carefully regulated and controlled by repeated blood examinations at different periods and requires special skill on the part of the radiologist. Moreover it might be very expensive for the ordinary patient for the course has to be repeated. Irradiation of the pylorus has no effect.

(4) Splenectomy. This is irrational and may be fatal.

After reviewing the above methods of treatment it seemed logical and reasonable to try and produce an anaemia which is not dangerous in the type of patient who feeds well, and which might be interrupted at will by inducing a helminthic anaemia by means of artificial infection with *Ancylostoma duodenale*. This new method has the following advantages (1) *Ancylostoma* infection can be easily produced in tropical and subtropical countries. (2) One or two inductions may last for a long time as *Ancylostoma* worms are not short lived parasites. Moreover *Ancylostoma* anaemia does not recover spontaneously. (3) If iron therapy is withheld no appreciable increase of haemoglobin occurs on normal

diet (4) *Ancylostoma* infection produces no lasting complications in adults. The patient need not be under strict medical supervision during the treatment and later on only a few blood counts are necessary. (5) *Ancylostoma* infection, unless the anaemia becomes excessive (less than 60 per cent Hb), needs no treatment as the infection is gradually eliminated. (6) Even after complete elimination of the infection the anaemia remains more or less stationary unless it is helped by iron therapy, or improves on full diet only very gradually. (7) If any untoward symptoms develop the parasites can be easily removed. (8) *Ancylostoma* anaemia is a hypochromic anaemia and patients can live without symptoms of anaemia at comparatively low levels of haemoglobin (60 to 70 per cent), since the red blood corpuscles are usually of normal number with this percentage of haemoglobin. The reduction of r b cs is more important than that of the haemoglobin in the production of symptoms of anaemias. (9) Such a method of treatment allows a patient to live happily for long periods away from skilled medical supervision. Although this patient was a hospital case, yet the expenses of this method would be much less than those of any of the methods discussed.

At the time of writing the patient feels and looks better. There is no giddiness, no headache, no palpitation on exertion, his pulse rate is 75 per minute, and he can walk better and for longer periods. His colour is normal, there is no congestion of the skin or engorgement of the face, the eyes are not bloodshot and the ears are not cyanosed as before. The throat is normal, showing no redness. He is now more energetic and does his regular job efficiently, although he feels unfit for prolonged work. His weight is still low. The spleen is enlarged + 2 fingerbreadths and rather tender.

The patient is being kept under observation.

COMMENT

An account is given of the treatment of a case of polycythaemia vera by induction of an artificial infestation with *Ancylostoma duodenale* in the patient. The progress of the case is recorded and the relative methods of this and other methods of treatment are discussed.

This is the second case of polycythaemia vera thus treated to be reported on, the first being that of DUVOIR *et al* of a woman in Paris.

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STUDIES IN LEISHMANIASIS IN THE ANGLO-EGYPTIAN SUDAN

X AN INTERESTING STRAIN OF LEISHMANIA *

BY
R KIRK, M D,
Stack Memorial Research Laboratories, Khartoum, Anglo-Egyptian Sudan

A previous communication in this series (Kirk, 1945) described a curious condition of cutaneous and mucocutaneous leishmaniasis in a monkey (*Cercopithecus aethiops*) which had been infected intraperitoneally with Sudan kala azar known as espundia, which is typically a clinical manifestation of American leishmaniasis.

This condition has not previously been observed in monkeys infected with Sudan kala azar and is probably of rare occurrence. It might easily have been missed in this instance owing to the long incubation period, and the fact that the writer was preparing to go on leave within a week at the time the nature of the condition was recognized. It was recognized that this was a very interesting observation, and that attempts should be made to follow it further. The obvious line of action was to continue passage of the strain through monkeys and note the type of infection which resulted. Fortunately, a number of monkeys of the same species, *Cercopithecus aethiops*, was available. Parasites from the infected monkeys were inoculated into four of those, each of which received a heavy inoculum on two occasions.

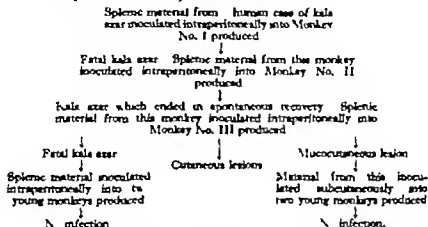
The infected monkey was anaesthetized with ether on the 19th February, 1944, its abdomen opened and a drastic form of splenic puncture, amounting in fact to spleen biopsy, was done. The material thus obtained was inoculated intraperitoneally into two of the new monkeys. A small residue was available for microscopical examination, and this revealed a heavy leishmania infection. At the same time a small piece of tissue was excised from the oro-nasal lesion, ground up in saline and inoculated subcutaneously into two other new monkeys. Owing to the likelihood of secondary infection, it was thought undesirable to inoculate material from this source into the peritoneal cavity. In this case also there was sufficient residue for microscopical examination, which revealed abundant leishmania parasites.

The procedure was repeated 4 days later on 23rd February, 1944. Splenic material was inoculated intraperitoneally into the same two monkeys as on the previous occasion. The other two received, as before, subcutaneous inoculation of material from the oro-nasal lesion. Microscopical examination showed that on the second occasion also all four animals received a heavy dose of parasites.

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The infected monkey from which these inocula were derived, died approximately a month later when the writer was on leave. This was expected. During the following 4 years the four monkeys which were inoculated with material obtained from this animal have shown no evidence of any type of leishmaniasis infection, and the strain has been lost. Three years after inoculation with material from the monkey with metastatic cutaneous and mucocutaneous leishmaniasis it was concluded that attempts to infect these four monkeys had failed, and that they could be used for other purposes as the laboratories were finding it difficult to obtain an adequate supply of monkeys. Within the last year all of them have died of other causes, and postmortem examination revealed no evidence of leishmaniasis infection.

The history of the strain may be summarized as follows:



COMMENTS

Cercopithecus aethiops is somewhat variable in its reaction to inoculation with *Leishmaniasis*, but as a rule it is easily infected by intraperitoneal inoculation of splenic material from the visceral disease. The course of the infection is very similar to kala azar in the human subject, generally ending fatally but sometimes ending in spontaneous recovery. LAYRAN (1917) stated that *Leishmaniasis* rapidly loses its virulence on passage in monkeys, but this assertion was based on slender evidence. In the present instance the conditions under which the transfer of infected material from Monkey No. 3 was made were such as to render unlikely the loss of the strain from purely accidental causes. Four monkeys were inoculated, all young animals, thus reducing the possible effect of individual resistance or immunity and the inocula contained large numbers of parasites.

It is concluded that the strain lost its virulence for monkeys with serial passage in these animals. The interesting point is that in so doing it passed through a stage in which it produced metastatic cutaneous and mucocutaneous lesions.

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OBSERVATIONS ON THE EPIDEMIOLOGY OF INFECTIONS WITH *CLONORCHIS SINENSIS*

RUDOLF P. KORNIGSHAIN M.D.
Kirang-Chu Hospital Hargchow, Chekiang China

The epidemiology of infections with *Clonorchis sinensis* has been amply discussed in most textbooks of tropical medicine and parasitology. The disease, as normally seen, is a predominantly chronic one. Normally the infections are found in Cantonese and Japanese, due to their predilection for raw fresh-water fish, the second intermediate host of this trematode. Symptoms are vague and indefinite: irregular appetite, fullness of the abdomen, diarrhoea, attacks of slight jaundice, only in a few cases severe consequences, such as chronic proliferative cholangitis and cholecystitis or portal cirrhosis were observed. These changes have been explained by Laster (1945) to be due to the permanent irritation of the biliary ducts by the worms, their eggs and their toxic products. Acute symptoms have not been described in Japanese or Chinese.

It seems of great interest to report an epidemic of clonorchiasis in Europeans, and to compare the symptomatology in Europeans with that in Chinese. While the symptoms in Chinese, as I have observed them, conform to the classical picture, the infection in Europeans gives a greatly different picture.

During the recent war a community of nearly 20,000 displaced persons of European origin, mainly Jews, accumulated in Shanghai. This group of people was isolated during the Pacific war from the other population in a small, overcrowded and unhygienic district of Shanghai (near the wharves), and the great majority were found to be in poor physical condition at the time of their liberation in August, 1945. Many of them had all through the war infections with gastro-intestinal parasites, ascariasis was predominant, nearly 50 per cent of the people had one or more attacks of this worm infestation. Nearly 20 per cent of this group had one or several attacks of bacillary dysentery, nearly 10 per cent attacks of infection with *Entamoeba histolytica*. Infections with *Giardia lamblia* varied round 10 per cent. Most people showed signs of chronic malnutrition and hypovitaminosis. The general nutritional situation was greatly improved after the liberation but the frequency of intestinal infections remained the same, due to the unimproved hygienic situation and the impossibility of finding adequate housing for so large a group in the already overcrowded city of Shanghai.

In February and March, 1946, a sudden epidemic outbreak of clonorchiasis was observed in this group. The source of infection was pickled fresh-water fish which were sold as herrings. These "pseudo-herrings" were bought

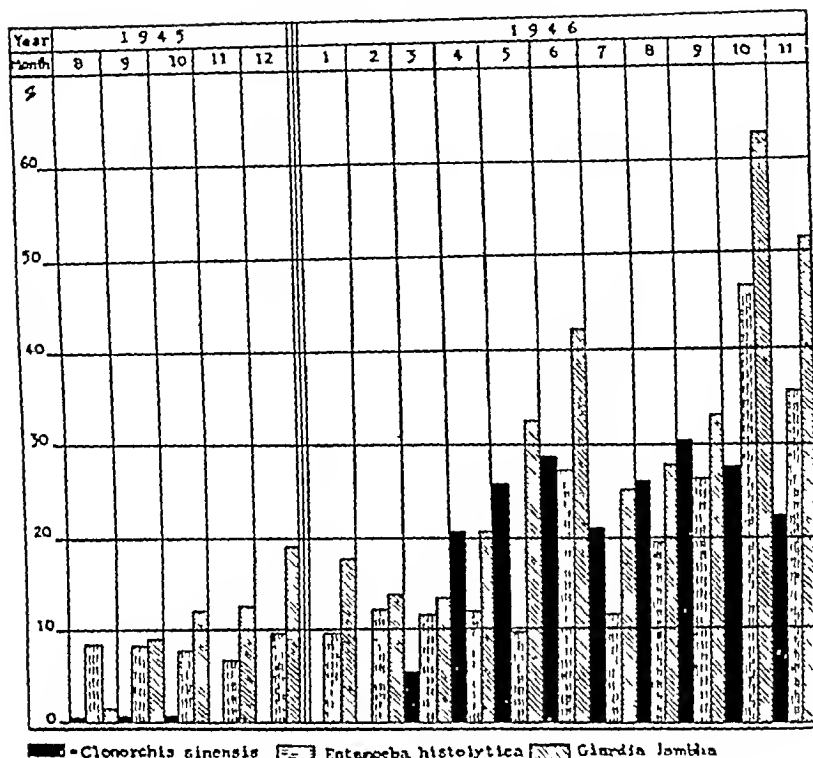
and eaten by many of the displaced persons, pickled herrings being a favourite dish of Germans. These fish appeared first on the market in the last weeks of January 1946. In February the first cases of clonorchiasis were observed. It seems that about 20 to 30 per cent of all displaced persons were infected.

In order to determine the percentage of the *Clonorchis*-carriers in the whole group of displaced persons, I asked the members of two boy-scout and guide troops, formed by Austrian and German refugees, to bring their faeces for examination. Only a single specimen from each member was examined. Fourteen positive cases were found in a total number of 64 persons. If each had sent several samples, the percentage of positive findings would have been still greater.

The clinical picture was that of an acute infection. The patient started with general malaise, subfebrile or febrile temperature, in a few cases onset with chills and fever up to 40° C. In most of the cases a slight subicteric tint was found in the sclerae and in the skin, the liver was enlarged and tender there was no hydrops of the gall bladder but a marked tenderness in the right ninth or tenth intercostal space in the back. Some patients had a slight or marked enlargement of the spleen. The blood examination showed a slight leucocytosis and an eosinophilia, on an average of 10 to 40 per cent in a few cases even up to 80 per cent. The highest eosinophil count observed was 83 per cent. The faeces examinations were in all cases negative for the first few weeks only 3 to 4 weeks after the onset of the disease did the faeces examination or the examination of the duodenal juice reveal the presence of *C. sinensis* ova. This late appearance of ova in the faeces made it impossible to recognize the first cases. The high eosinophilia naturally pointed to a parasitic disease, a Loeffler syndrome being excluded in the absence of signs of disease of the respiratory tract.

It now seems clear that the first symptoms were due to the invasion of the biliary passages by the metacercariae. About 1 month later after the development of the metacercariae into adult worms ova could be found in the faeces. Most patients when specially questioned said that they had eaten these so-called herrings a few days before the onset of the disease. I examined three of these fish and in two instances I was able to find metacercariae in their dorsal muscles. The disease was self-limited after a few weeks the acute symptoms subsided, but general malaise remained in a great majority of the cases, the symptoms resembling more the classical description of clonorchiasis. With the gradual subsiding of the acute symptoms, a parallel decrease of the eosinophilia was observed. In the chronic stage, counts of between 5 and 10 per cent of eosinophiles were found in the blood. The level of the eosinophilia was definitely not related to the number of ova which could be found in the faeces it was more or less parallel to the severity of clinical symptoms, and thus might be taken in these cases as an indicator of the allergic reaction of the host to the parasite.

The coincidence of infestation with *C. sinensis* and diabetes has been



Each block represents the percentage of positive findings in the total number of faeces examinations made in each month

reported in several cases MUTO-NARDONE (1946) and MANSON-BAHR (1947) reported a few such instances In this epidemic a few cases of this kind were observed by TENNENBAUM TENNENBAUM, on the other hand, found a few examples of hypoglycaemic attacks in patients infected with *C. sinensis* I myself observed one patient with attacks which seemed to be of the nature of *petit mal* and first started after the onset of the epidemic of clonorchiasis A faeces examination revealed the characteristic ova, a glucose tolerance test showed a very low hypoglycaemic phase (lowest point, 40 mg per cent. 2 hours after the intake of dextrose gramme 60, the postprandial hyperglycaemic phase of the tolerance test being normal) These observations regarding carbohydrate metabolism are too limited to indicate any definite association between clonorchiasis and disturbance of the insular apparatus, but further research in this direction seems promising

One of the most prominent features of this epidemic was the simultaneous increase of intestinal infections The data given in the appended table were collected in my former private laboratory in Shanghai, beginning in August, 1945, and ending in November, 1946 The total number of faeces examinations in these 16 months was over 3,500 The table very clearly shows the increase of infections with *G. lamblia* and with *E. histolytica*, which followed the onset

of the clonorchiasis epidemic. It seems that the infestation with *Clonorchis* lowered the resistance of the individual to other parasitic diseases of the intestinal tract, and thus the spreading of the ubiquitous amoebae and *Giardia* was made possible. While the infection with *Clonorchis* cannot be spread without eating raw or insufficiently cooked fish, the infections with amoebae and *Giardia* are spread by flies, cats, dogs and from man to man. That seems to be the reason for their predominance over the *Clonorchis* cases in the later phase of the epidemic, when new infections with *Clonorchis* were prevented by adequate warning of the population concerned against eating these fish.

Therapeutically this epidemic did not bring out any new facts, the only medicine which seemed to alleviate the symptoms and to reduce the number of ova in the faeces for some time at least was gentian violet. Treatment with antimony compounds seemed completely without success, so far as the number of ova in the faeces was concerned. The simultaneous infection with *Clonorchis*, *E. histolytica* and *G. lamblia* in a great proportion of the cases provided an especially difficult therapeutic problem. The amoebae and *Giardia* seemed much more resistant to the usual methods of treatment. In several cases simultaneous medication with emetine, yarene, carbasone and atebine proved to be successful.

This epidemic brings forward two rather interesting points: first the clinical picture of acute clonorchiasis, a syndrome which up to now has been very rarely seen. The second and more important point is that this epidemic took place in a population group which up to now has not been infected with this trematode. Chinese, especially Cantonese and Japanese have harboured this infection for centuries. The fact that an acute outbreak of clonorchiasis has never been observed among them seems to prove the assumption that they have developed a relative immunity against this trematode. In Europeans, no immunity against *Clonorchis* exists and therefore the symptomatology of the infection was greatly changed. The question of immunity against helminthic infestations has been widely discussed. The experiences in the epidemic now described, with the very marked racial differences in the reaction to this trematode infestation, make it almost necessary to assume some kind of relative racial immunity to special kinds of helminthic infections.

SUMMARY

An epidemic outbreak of clonorchiasis in a group of European displaced persons in Shanghai is reported. Clinical and epidemiological details are given, and the question of racial differences in the immunity reaction to this infection is discussed.

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CORRESPONDENCE

MECHANICAL PURIFICATION OF RIVER WATER

To the Editor, TRANSACTIONS of the Royal Society of Tropical Medicine and Hygiene

SIR,

It has seemed to me that the chances of contracting schistosomiasis are much reduced when water is pumped through metal piping, especially where it is passed through more than one tank and drawn from the bottom layer rather than the surface which harbours those cercariae which attack man

Water which I obtained from a polluted stream at the same time as I collected water which had been pumped up 250 feet, was reported by Dr NORMAN WALKER, of the Natal Pathological Laboratory, to show distinctly more *Bacillus coli* than that which had passed through the pump, and no snail hosts were found

Dr WALKER then examined for me some water obtained in the early morning from an open stream near Kearsney College and from its swimming bath, after being pumped up 600 feet and before any chemicals had been added. Here again there was distinctly less *Bacillus coli* infection in the pumped sample.

A colleague has kindly supplied me with some unpublished results he has obtained for me in this connection showing the effect of a pumping system on river water which could not be attributed to the mere storage of water, by which means both enteric and schistosome infection are known to be reduced.

Raw river water at the inlet to the first pump revealed 1,200 coliform organisms per 100 c.c. At the discharge from the first pump and inlet to the second pump it contained 600, that is after being raised 250 feet, whilst at the inlet to the third pump after being raised another 250 feet it contained but 350. At the outlet to the third pump and inlet to the purification plant, after being raised approximately 750 feet in all, the water contained 350 coliform organisms per 100 c.c., and the question arose how far similar improvement might occur without raising it to great heights.

Dr C. A. M. MURRAY kindly supplied me with the result of testing water from the Little Umalindusi River at Inyoni and after it had been pumped over a distance of about three-quarters of a mile, raised about 100 feet, and transferred to a second tank which was emptied about every 24 hours. In this case the raw river water contained 160 of the intermediate coli aerogenes group of organisms per 100 c.c. a differential test showing the faecal type of *B. coli*, whilst the tank water contained only 40 per 100 c.c., the differential test also showing the faecal type of *B. coli*.

When estimating the amount of chemical substance required to purify a town's water supply account must be taken of the chemical analysis and pathological findings of reservoir water rather than those of the rivers from which it is derived. For purifying the 22 million gallons of water supplied to Durban daily from the Umgeni river £13 000 worth of sulphate of aluminum is required annually.

If not exposed to pollution, flowing water tends to purify itself by contact with stones in river beds and by passing over waterfalls but rushes and the molluscan hosts of trematode parasitic worms tend to accumulate at the foot of waterfalls and re-infect clean falling water.

I am, etc.

F GORDON CAWSTON

Britannia Buildings,
Durban.

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A CASE OF RECURRENT HAEMOGLOBINURIA TREATED WITH SULPHATHIAZOLE

SIR,

A specimen of urine, from a case of haemoglobinuria—in an Indian girl aged 6 years—was sent to the Ft. Johnston Hospital, Nyasaland, for examination during August, 1947. The blood was positive for subtertian malaria and streptococci were found in the urine. She was treated with paludrine and sulphathiazole, grammae 0.25 t.i.d.

There followed an alternating absence and return of haemoglobin until the fourth day when it disappeared. Probably the dose of sulphathiazole was not large enough to effect early control. From that time the child kept well until the morning of the 19th November 1947 when the father noticed again

the passage of dark-coloured urine, and brought her to hospital (walking) at 10 30 a m. The temperature was 98 2° F, the blood negative, and, although the girl looked pale, there were no obvious signs of illness. The urine was of a dark port-wine colour, and a microscopical examination showed streptococci.

Sulphathiazole, gramme 0.5, was given at 10 45 a m, to be repeated 4-hourly.

The father was instructed to send a specimen of the urine by 12 noon, and the specimen, which must have been passed about 15 minutes before, was received at 12 15 p m at the hospital. The colour had changed to a medium amber, suffused with a rosy tint, 1½ hours after the first dose of sulphathiazole. The next day, the 20th, the early morning urine was smoky, but a specimen passed at about 10 a m was clear, and the urine remained so thereafter.

The patient was not under antimalarial prophylaxis before or at the time of the attack, and no antimalarial drug was given during the illness.

The sulphathiazole was continued until the 24th November, when the patient ceased attendance.

I am, etc,

Ft Johnston,
Nyasaland

J O SHIRCORE

8th March, 1948

INTESTINAL INFECTION OF PRIMATES

SIR,

In your issue of January, 1949, Dr R E REWELL comments on an apparent error in my article on *Balantidium* infestation in primates. In this it was mentioned that *Shigella flexneri* could cause diarrhoea, and Dr REWELL is of the contrary opinion. In support, he quotes an outbreak in the Zoological Gardens, Regent's Park, reported by Colonel BRIDGES and himself in Bulletin of the Public Health Laboratories for January, 1948, which states that no signs of diarrhoea were noted.

It so happens that Dr REWELL and I are referring to the same outbreak, as at that time he was Pathologist to the Society, while I was in charge of the health of the animals in the menagerie. His outline of the epidemic is incorrect, probably because he saw only the monkeys that died, the survivors being strictly isolated as soon as the outbreak was discovered.

The full story is as follows. Sixteen Rhesus monkeys were kept together in a large cage near the rodent house. One was found dead one morning, and Dr REWELL later reported *Sh. flexneri* present. A few mornings later all the

disappearance of the disease from the smaller towns and villages in the surrounding countryside. From this it was concluded that the endemic foci were confined to a limited number of the larger cities, or key-centres, and it was confidently predicted that the control of the vector in these foci would eventually result in the complete eradication of the disease. However it was found, in Brazil, that yellow fever did not disappear after many years of effective control in the principal cities, and it became evident that the key-centre theory was not applicable in all areas, and that it could not be made the basis of a successful eradication campaign.

The first indication of the existence of epidemiological factors which had not previously been taken into account came with the report by SOPER *et al.* (1933) of an epidemic of yellow fever in an area in Brazil in which *A. aegypti* did not exist. Since then, many outbreaks of the disease have been observed in *aegypti* free areas in South American countries, and it is now known that yellow fever is endemic and widespread in sparsely populated regions, under conditions which indicate that neither man nor the mosquito, *A. aegypti*, are essential elements in the insect vertebrate cycle of infection. This type of the disease, which has become known in the Western Hemisphere as "jungle yellow fever" has been fully described in the literature, and it was discussed at a meeting of this Society by Dr F. L. SOPER (1938), over 10 years ago. At that time it was not known whether an epidemiological type of yellow fever analogous to jungle yellow fever exists in Africa, but an intensive study which was commenced in an area in Central Africa, in 1937 and which is still in progress, has provided the answer to that question. The detailed results of this investigation have already appeared in a series of publications and an attempt will now be made to review and summarize them.

The demonstration by STOKES *et al.* (1928), and by THILLER (1931), that the virus of yellow fever can be established in laboratory animals was quickly followed by the perfection of the mouse protection test (SAWYER and LLOYD, 1931). This test made it possible for the epidemiologist to study the distribution of immunity to yellow fever on a large scale by means of regional surveys. An immunity survey which included practically all parts of Africa, was carried out, and the results revealed that there is a wide belt of immunity extending from the West Coast through Central Africa into Uganda and the Anglo-Egyptian Sudan (SAWYER and WHITMAN 1936). In the western portion of this area many cases of yellow fever had been observed and virus had been isolated from several of them. In the eastern section of the belt of immunity on the other hand, the disease had never been recognized, apart from a single case (HEWER, 1834). The absence of observed cases in this vast area led some persons to doubt the validity of the protection test, while others believed that if, as indicated, the disease had been widespread, it must have occurred in an unrecognizable form. In the hope of finding the answer to some of the questions raised by the immunity survey in Central and East Africa, a Yellow Fever

Research Institute was opened in Uganda late in 1936 * During the early work of the Institute, attention was focused on an area in western Uganda, known as Bwamba County, when a preliminary survey revealed a relatively high incidence of immunity to yellow fever, and further investigation pointed to this as the most suitable area for long-term epidemiological studies Work was commenced in the area in 1937 and, except for a short break in 1940, has been continued to the present time In the meantime, any doubts which existed about the occurrence of yellow fever in Central Africa were removed, in 1940, when the most extensive epidemic which has ever been reported anywhere in Africa occurred in the Nuba Mountains region of the Anglo-Egyptian Sudan (KIRK, 1941) During the course of this outbreak two strains of yellow fever virus were isolated and studied in the laboratory The results indicated that they were in every respect similar to strains previously isolated in West Africa and in South America (MAHAFFY *et al*, 1941)

Our conception of the epidemiology of yellow fever in Central Africa is based, almost exclusively, on the results of the investigations which have been carried out in Bwamba County This is a small, heavily-forested area which lies in the extreme west of Uganda between the north spur of the Ruwenzori Mountain Range and the Semliki and Lamia rivers, on the Uganda-Congo border (Map 1)† The northern portion of the county is occupied by the Semliki Forest, an eastward extension of the great Ituri Forest of the Belgian Congo This forest has been designated a sleeping sickness area and is closed to human habitation It contains large tracts of swamp and high bush, but in the main, it consists of dense primary rain-forest Between this forest and the mountain there is a cultivated zone with approximately 35,000 inhabitants (Map 2) A detailed description of the area has been published by HADDOW (1945a), to which reference should be made for further information on topography and vegetation

When work was commenced in Bwamba in 1937, it was a relatively inaccessible area which could only be reached on foot by a path leading over the mountain Work had, however, begun on a road which, ultimately, would join county headquarters in the inhabited zone with the network east of the mountain After emerging from a low pass at the north end of the mountain, the projected road traversed several miles of uninhabited forest, before bearing left into the cultivated area The labourers engaged on the construction of the forest section of the road were kept under observation over a period of approximately 1 year, and those who exhibited febrile illness, without obvious cause,

* This Institute was originally supported jointly by the Rockefeller Foundation and the Government of Uganda More recently it has been financed by grants made by the Rockefeller Foundation and by the Secretary of State for the Colonies under the Colonial Development and Welfare Acts, and by contributions from the Governments of Uganda, Kenya, Tanganyika, Northern Rhodesia, Nyasaland and Zanzibar

† I am indebted to Dr A C LOVETT-CAMPBELL, late of the Colonial Medical Service for the preparation of Maps 1 and 2

were bled and their sera inoculated into white mice. A new clinical entity was studied and its causative virus was isolated (SMITHBURGH *et al.* 1941), but the presence of yellow fever was not detected. A possible explanation for this negative result became apparent during the course of the studies which were to follow and it will be referred to later.

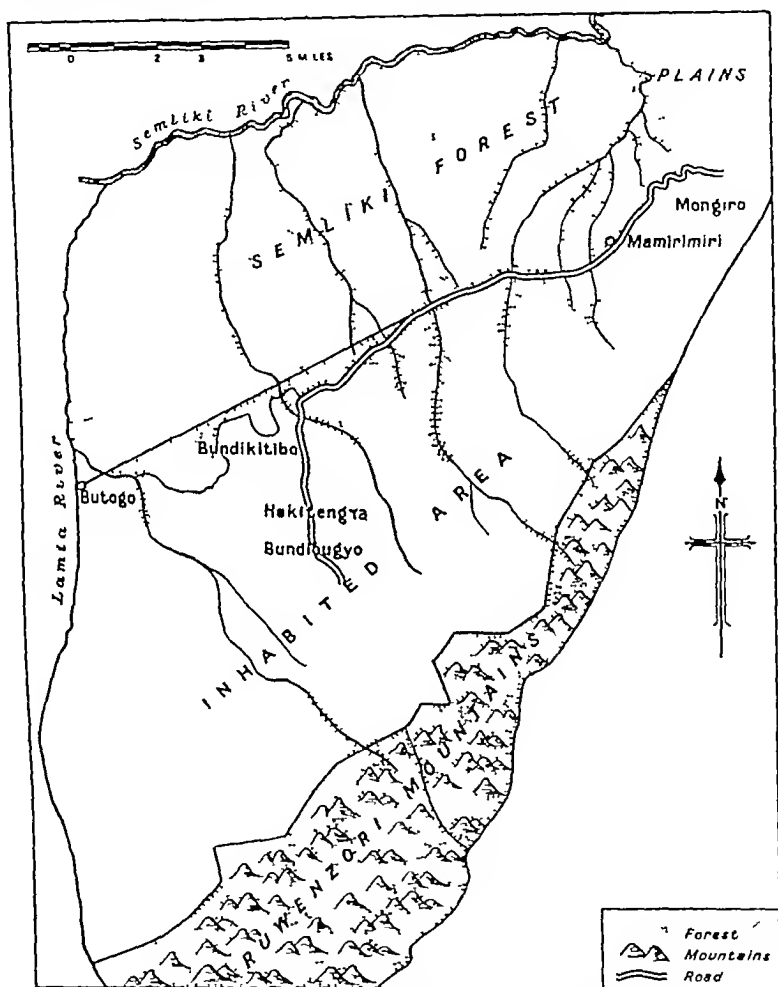
Coincident with this attempt to isolate virus from human cases in 1938, a preliminary mosquito survey of the area was undertaken by Mr J. O. HARPER,



MAP 1. MAP OF AFRICA
(A. ow sh w l t i f Bw uba C nty Ug ad)

whose services were made available by the Kenya Medical Department. He collected much useful information on the numerous species met with in the forest as well as in the cultivated area. He noted, for example, that, although the classical vector of yellow fever *Aedes aegypti* was present, it did not occur in sufficient numbers to enable it to play a role of any importance in the transmission of the disease. On the other hand, *Aedes (Stegomyia) impavens* Theob. was the only species that could be captured in large numbers during the daytime in the inhabited zone. The possible importance of this potential vector was realized, and large numbers were caught and sent to the laboratory in an

unsuccessful attempt to isolate yellow fever virus. More detailed work on *A. simpsoni* was afterwards carried out by the late Mr E G GIBBINS (1942), of the Uganda Medical Department. He collected and examined large numbers of *A. simpsoni* larvae and demonstrated that, in Bwamba, this species breeds, by



MAP 2 MAP OF BWAMBA COUNTY UGANDA

preference, in the axils of a particular variety of banana, in colocasias and in pineapples. Extensive studies, carried out at a later date by HADDOW (1945c), confirmed the earlier findings and added much additional information. Briefly, he showed that *A. simpsoni* is almost exclusively diurnal in its habits, that it frequents not only banana but other plantations as well, that it enters the edge

of the forest, and that it appears to prefer human blood although it will also bite monkeys. By a series of 24-hour catches carried out, simultaneously at ground level and on platforms at 6, 12 and 18 feet, it was shown that it bites at all levels, a point of considerable importance, as will be seen later. Incidentally these low platform catches provided useful training for the African boys who were to take part later in catches on very much higher trees in the forest.

An intensive immunity survey of the Bwamba population, carried out during 1939 showed that while immunity was widespread it was unevenly distributed (HUGHES *et al.*, 1941). Immunity among adults was found in many parts of the area. The percentage of immune adults was very low on the mountain slopes, and in the lowlands it rose steadily as the forest was approached, and reached its maximum in areas adjoining the forest boundary. Immunity in children was largely restricted to districts adjoining the uninhabited forest. The connection between the forest and yellow fever immunization of the human population was, therefore, quite definite (MAHAFFY *et al.*, 1946).

A concentrated study in two small areas in the zone of high immunity during the month of April, 1940 did not reveal any illness clinically resembling yellow fever nor was the virus of that disease isolated. However periodic examination of a selected group of non-immunes resident in this zone demonstrated that 48 of 168 of these individuals had been immunized against yellow fever between October 1939 and June 1941. As soon as it became known that the disease had actually been present in Bwamba within recent months, an investigation was at once undertaken to determine whether the virus was still active. This investigation, which included clinical epidemiological and entomological studies, was concentrated in the six local districts where residents were known to have been recently immunized. During the course of this brief study two cases were seen in which a clinical diagnosis of yellow fever seemed justified, and a strain of virus was isolated from one of them. The entomological study quickly confirmed the earlier observations on the mosquito, *A. simpsoni* and pointed to it as the vector most likely to be concerned in the transmission of the disease. It is a "semi-domestic" mosquito which breeds profusely in Bwamba, in plant axils in the vicinity of human habitations, and it was known to be a potential vector of yellow fever (PHILIP 1929). Large numbers of females of this species were captured in plantations, near houses where cases were under observation or had recently occurred, and sent to the laboratory. Two strains of yellow fever virus were isolated from these wild-caught mosquitoes. The data obtained from this study indicated that an epidemic of some magnitude had occurred in the area—that the outbreak in the human population was associated with contact with the forest—and that once it became established in man the principal vector of the disease was *A. simpsoni* (MAHAFFY *et al.* 1942).

As a result of the isolation of virus, the Uganda medical authorities carried out a mass vaccination of the entire population, in the hope of preventing east

spread of the infection. However, the epidemiologist and we continued and an essential feature of the work at this time was an attempt to determine whether yellow fever virus could persist in the absence of a susceptible human population. With this in view, catches of 4 mosquitoes were returned in plastic bags near the forest edge and three numbers were sent to the laboratory for inoculation into man. A run of yellow fever virus was again isolated from the species in July, 1942 (Sutcliffe and Hwang, 1943). This isolation experiment, as it did nearly 1 year after the completion of the numerous campaigns, implied the persistence of the virus in a "catcher" rather than in a prey animal in the forest edge of the forest.

Victims were now detected mainly as a result of the mosquitoes and the removal of the uninfected forest. Mosquito catches on a large scale were organized and systematic surveys of the area were made and the preparation of more detailed maps combined with exploration of the forest and the preparation of maps. The great profusion of the species encountered made for additional difficulty, especially during the early stages of the work. The extent of the problem is indicated by the fact that the species list from the study area in Iwamba includes about one quarter of the known Ethiopian fauna to date. The mosquito catches gradually extended along the forest edge and into the made for a shield as the Semliki River. Between July, 1942 and early April, 1944 nearly 70,000 forest mosquitoes were collected and identified and the majority of them were inoculated into laboratory animals in an attempt to isolate virus. In view of the large number of species involved it was impossible to deal with each separately and groupings of species had to be employed. Yellow fever virus was not isolated but much valuable information about the forest and its mosquito fauna was accumulated.

While the routine catches mentioned above were in progress a special investigation was undertaken to determine the daily biting cycle of the more important species and to find the best method of capture. Climate observations were made at the same time with the object of learning whether or no the biting behaviour of the species concerned could be explained in terms of the micro-climate of the habitat. A series of 15 controlled catches each lasting 24 hours was made in the forest, and another series of 15 in banana plantations. The catch for each hour was recorded separately. The results gave useful information on the biting cycles of almost all the common species and provided a basis for future work directly concerned with the search for yellow fever virus. It was shown that many forest mosquitoes occur in banana plantations, and an analysis of the day and night catches suggested that they migrate into plantations from the forest during the night. The climate observations indicated that, while the forest was cooler and moister than a banana plantation by day, the two environments were climatically very similar at night. Thus the micro-climatic barrier, which restricts the movements of the sylvan mosquitoes, is removed at night, permitting them to leave the forest. This finding has a bearing on the

yellow fever problem since it suggests one method by which the virus might be conveyed from the forest to man. For details of this important study the publication by Hadow (1945b) should be consulted.

Let us now consider briefly the animal studies which were carried out concurrently with the mosquito work. For the purpose of this discussion, the most important feature of this investigation was an extensive immunity survey in which large numbers of blood specimens were collected from many different species of animals. Immunity to yellow fever was found only in wild primates, mainly monkeys. Sera obtained from monkeys in the Ruwenzori foothills were all negative, a finding in accord with the very low incidence of immunity among humans in the mountain area. In the Semliki Forest, on the other hand, the results showed that nearly 60 per cent. of the monkeys were immune to yellow fever. Ten of the 12 species represented in the lowland forest were included in the survey and immunity was found in all of them. Statistical analysis of the results by species, sex and locality failed to show significant differences, but analysis by age grades revealed that the incidence of immunity increases steadily with increasing age, reaching a maximum of nearly 90 per cent. in "old" monkeys (A. J. Hadow *et al.*, 1947b). This observation strongly suggests that yellow fever is enzootic in the monkeys of the lowland forest. Of the various species, some are terrestrial in their habits, some are mainly arboreal, and others are almost exclusively arboreal. The occurrence of a high immunity rate among the strictly arboreal species would indicate that the forest vector must have pronounced arboreal tendencies, while, among monkeys that are mainly terrestrial, it would suggest either that the vector must be equally prevalent in trees and at ground level or that it must bite at night when all species are asleep in the trees. At about this time, the first hint that arboreal mosquitoes might be involved came from South America, when it was learned that a tree haunting species, *Haemagogus capricornis* had been found to be an important vector of jungle yellow fever in Colombia (Bucñez *et al.* 1944).

Although it was now known that monkeys were involved in the forest cycle, there was still nothing to indicate what vector was responsible for the transmission of the virus from animal to animal. It was, however possible to lay down the following postulates with regard to the forest vector: (a) it must be capable of transmitting yellow fever virus; (b) it should be present throughout the year; (c) it should have marked arboreal tendencies; (d) it should have a wide forest distribution; (e) it should be a prevalent species; and (f) it should be a species which bites at night.

The search for the forest vector was continued. The fact that no significant differences were found between the immunity rates in monkeys in different localities indicated that an intensive programme might be confined to a single location. For various reasons, this was much to be preferred to a study embracing a wide area. A narrow strip of heavy rain forest (Mongiro) was, therefore selected and large-scale mosquito catches, in 1944, were at first confined to this area. One such catch, made late in April, contained 80 *Aedes* belonging to 12

different species, but including no *A. aegypti* or *A. simpsoni*. From this lot of forest *Aedes* a strain of yellow fever virus was isolated, a finding which provided proof of the existence of a forest cycle of yellow fever in an area uninhabited by man (SMITHBURN and HADDOW, 1946).

Of the 12 species in the infected lot of 80 *Aedes*, 10 could be eliminated from serious consideration on grounds of scarcity, restricted distribution, or because they were almost exclusively terrestrial. Only two species remained, *A. africanus* and *A. apicoargenteus*. The latter had been found by BAUER (1928) to be incapable of transmitting yellow fever virus, and this was confirmed in a test in the Entebbe laboratory. The other species, *A. africanus*, was known to be a good vector under laboratory conditions, and it has a wide distribution in the African forests. It was, therefore, highly suspect.

It was now apparent that a study of the arboreal mosquito fauna in the Semliki Forest must be undertaken. In order to gain the maximum information on the vertical distribution of mosquitoes in the forest, a series of 24-hour catches was carried out, simultaneously, at ground level and on tree-platforms at heights which varied from 16 to 82 feet above ground level. The platforms were constructed in carefully selected trees in two adjacent forest areas (Mongirola and Mamirimiri), and were reached by ladders attached to the trees. The mosquitoes were captured while attempting to feed, and the work was organized in such a way that the catch at each station for each hour of the day and night could be recorded separately. An original series of 10 catches was made in each area, and this was repeated some months later at the end of an exceptionally long dry season.

When the results of the two series of 24-hour catches were analysed, they provided a mass of information on the biting habits of the various species, as well as on their prevalence at the different levels (HADDOW *et al.*, 1947a). The main arboreal species were found to be *Aedes (Mucidus) grahami*, *Aedes (Finlaya) longipalpis*, *Aedes (Stegomyia) africanus*, and various species of *Taemorrhynchus*. Of these *A. grahami* and *A. longipalpis* are of some potential interest, provided they can be shown to be capable of transmitting the virus. *A. apicoargenteus* does not appear to be able to act as a vector. The prevalence of *Taemorrhynchus* spp. in the canopy is of interest since one species (*T. africanus*) is known to be a potential vector (PHILIP, 1930). This mosquito is present in the canopy throughout the year and it has a wide distribution and long flight range. It bites freely at ground level and in trees, and enters houses in large numbers. It is believed, therefore, that it may well enter the epidemiological picture, particularly in the drier, savannah areas. The findings of greatest interest were, however, those concerned with *A. africanus*. This species was by far the most abundant arboreal culicine, attaining its highest concentration in the forest canopy. It showed a pronounced peak of biting activity in the hour after dusk, when all species of monkeys are in the trees and are quiescent after the evening meal. It is a good vector in the labora-

tory has a very wide distribution, and is present in the adult stage throughout the year. It, therefore, fulfilled all the theoretical postulates, and it was already under suspicion since it was represented in the lot of forest *Aedes* from which yellow fever virus was isolated (Hadow *et al.*, 1947a).

Since all the evidence pointed to *A. africanus* as the most likely forest vector the next step was a full-scale attempt to isolate yellow fever virus from this species in the field. It was decided to make the attempt in the same area in which the tree-top catches had been carried out. Additional platforms were built in the upper canopy at Monguro, and the old platforms in this area, and at Mamurimuri, were brought into use. Catches were made each evening for 5 or 6 days a week for 11 weeks. The results again confirmed that *A. africanus* is the predominant species in the canopy in the sunset period. In the hope of isolating virus from an infected mosquito by bite, all the *A. africanus* taken in these catches were given an opportunity of feeding on a rhesus monkey before they were ground up and injected into another animal. It is of interest to note that 85 per cent. of them actually engorged. Mosquitoes of other species were inoculated into rhesus monkeys by groups. Yellow fever virus was not isolated, but previous findings with regard to *A. africanus* were confirmed and much additional information was gained (Hadow and MURPHY 1947). The readiness with which this mosquito fed on captive monkeys raised the question of its behaviour in this respect in nature. Catches on high tree platforms were accordingly made anaesthetized African monkeys being used as bait: these showed that *A. africanus* not only feeds freely on these animals in trees but that it appeared to prefer them to human beings.

It was now apparent that, in the absence of definite evidence of virus activity in a particular area, large scale catches were not an economical method of searching for the forest vector. In an attempt to get this evidence, it was decided to expose rhesus monkeys to the bites of forest mosquitoes in the hope that one of them would contract the disease and thus indicate a focus of virus activity. The monkeys were confined in cages, the sides and bottoms of which were made of expanded metal, and these were placed on tree platforms in the upper canopy. In a preliminary trial four of these so-called sentinel monkeys had been exposed in the Monguro area. A more extensive sentinel programme was now initiated and 16 animals were placed in position on high tree-platforms along 10 miles of the forest edge. Temperatures were taken daily and each animal was bled monthly for an immunity test. This programme was fully developed late in 1945.

Next came the finding that children resident in a large area near the forest edge and born since 1941 when mass vaccination was carried out showed a high degree of immunity to yellow fever. This indicated that these susceptible human beings were being immunized by virus coming from the forest. As the area involved lay beyond that covered by the sentinel monkeys already in position, the number of these animals was considerably increased. Eventually

the entire forest edge from Mongiro to Butoga, as well as a number of selected banana plantations near the forest, were included in the sentinel programme. While these animals were being kept under observation, mosquito catches were continued in the forest as well as in banana plantations. Very large numbers of mosquitoes were captured on the platforms and at ground level and sent to the laboratory for inoculation into animals. Yellow fever virus was not isolated, nor did any of the sentinel rhesus monkeys contract the disease or become immune to it.

While this work was in progress, various factors, which might have a bearing on the continued failure to isolate virus, were also being investigated. This resulted, late in 1947, in the unexpected discovery that *A. africanus* did not readily enter the type of cage used for confining the sentinel monkeys. This meant that the cages were actually protecting the monkeys against the particular mosquito which all the evidence indicated was the most likely forest vector. The experiment was being doomed to failure because the temperamental female *A. africanus* would not venture into a wide open cage to partake of its evening meal. It was necessary, therefore, to reconstruct and rearrange the platforms in such a way that the monkeys could be accommodated on them without resorting to the use of cages. This work, which was commenced in October, 1947, was completed early in February, 1948, by which time all the cages had been discarded. Meanwhile, mosquito catches were continued both at ground level and on sentinel platforms in various localities. All the *A. africanus*, and many of the other mosquitoes, were inoculated into laboratory animals. Yellow fever virus was not isolated, but one lot of *A. africanus* captured in the Mongiro-Mamirimiri area in December, 1947, was shown to be infected with the virus since it immunized the rhesus monkey into which it was inoculated (HADDOW *et al.*, 1948). Events which were soon to follow provided additional convincing evidence of the important role played by this species in the forest cycle of yellow fever.

In June, 1948, it was found that one of the sentinel rhesus monkeys in the Bundikitibo area had become immune to yellow fever. The specimen of serum which gave the positive protection test result was taken on 3rd June. On 25th June another sentinel was found in a moribund condition. Yellow fever virus was isolated from the serum of this animal and the liver showed the characteristic lesions of the disease. During the next 4 months, six additional sentinel monkeys in the same area contracted the infection and yellow fever virus was isolated from three of them.

As soon as it became known that yellow fever was active in the Bundikitibo area, mosquito catches were commenced there. Some continuous 24-hour catches were made, simultaneously, in the forest canopy and at ground level, but the majority of them were carried out on sentinel-monkey platforms during the afternoon and evening period, at which time they could be expected to yield the maximum number of arboreal mosquitoes in which interest was mainly

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DISCUSSION

Dr G M Findlay It may not be without interest to Fellows of this Society to remind them that the modern conceptions of the epidemiology or perhaps one should say the epizootology, of yellow fever, is largely based on the observations of two Past-Presidents of this Society Before the first world war Sir PATRICK MANSON had suggested that other mosquitoes, in addition to *Aedes aegypti*, must act as vectors of the disease A few years later, Sir ANDREW BALFOUR, observing that red howler monkeys were dying in the forest immediately before the onset of an epidemic of yellow fever among men, suggested that monkeys played an important role in the spread of the disease

The first monkey in Africa to be found with immune bodies to yellow fever was shot in 1935 by Miss FANNY WALDRON in the forest region to the West of Kumasi in the Gold Coast, curiously enough, it was an entirely new species of monkey, a red *Colobus*, now known as *Colobus waldroni* By 1937 it was possible to show that monkeys with immune bodies to yellow fever only in West Africa but as far east as the Blue Nile in the forest region to the Sudan and as far south as Uganda and French Equatorial Africa

During the war years 1940 to 1945, more than 227,000 African soldiers were enlisted in armed forces of the Allies and some 50,000 European troops were stationed in West Africa There were no cases of yellow fever among African soldiers who were all inoculated against yellow fever, nevertheless, there were during the same period a number of cases epidemic in Nigeria and shortly after the end of the war a considerable epidemic in Sierra Leone and freedom from yellow fever of African troops is the more remarkable since they were engaged in training in all types of country, including primary forest, at all hours of the day and night Among the European troops there were three cases of yellow fever These occurred early in 1942 during a battalion exercise in light secondary forest not far from Allen Town in Sierra Leone It is of interest that an intensive mosquito survey in this area failed to show the presence of *Aedes aegypti* while an immunity survey of Allen Town, the nearest village, showed that no person under 16 years had immune bodies to yellow fever Monkeys in the area showed immune bodies A similar occurrence took place in the Gold Coast, a small girl, an inhabitant of Accra, visited her father's rice farm, situated in a village between N'swam and Swedru She contracted yellow fever and died A search for mosquitoes carried out by Major MATTINGLY showed an absence of *Aedes aegypti* and, in addition, no child under 10 years of age in the two adjacent hamlets had immune bodies to yellow fever White crested mangabeys from this secondary forest area in the Gold Coast, however, showed immune bodies One of the most remarkable and still unexplained findings in connection with the epidemiology of yellow fever is the presence of immune bodies in children in villages where there has never been any overt case of yellow fever Bakau, a village at Cape St Mary in the Gambia, has certainly had no known case of yellow fever, nor have there been

cases of the disease in the area, since the Bathurst outbreak at the end of 1934 yet some 25 per cent. of children under the age of 10 born in the village showed immune bodies to yellow fever. Similar findings were noted at Komenda in the Gold Coast. Komenda consists of two adjacent villages on the sea-shore with a hinterland of grassland and occasional scattered bushes. Until 1944 there had been no recorded case of yellow fever in the area then an African naval rating who had not been successfully immunized, succumbed to the disease and an investigation of the children under 10 years of age showed that again nearly a quarter showed immune bodies to yellow fever.

A full account of the investigations carried out on yellow fever in connection with the armed forces during the years of the second world war will be published shortly.

Mr P. F. Mattingly. It is a privilege to be asked to speak after Dr MAHAFFY whose work in Uganda has been an inspiration to all medical entomologists. The story which he had told us has been one of solid hard work meeting at last with its just reward and, like all the best stories of the kind it opens up new vistas and suggests many new lines of research.

It would be impossible to discuss these in detail in a short space of time, but they may perhaps be considered collectively in relation to the single problem of how far the discoveries made in Uganda may be expected to apply in other parts of Africa. To this problem three separate, though closely interrelated, lines of approach seem to be necessary. The first is the taxonomic and poses the question of how far the mosquitoes studied in Uganda can be regarded as the same as those found elsewhere. It is not a simple problem and the final answer can only come after intensive study and the accumulation of far more material from far more localities than is at present available. The second question is that of behaviour. Studies carried out in Nigeria on similar lines to those followed in Uganda have shown that the biting cycle and vertical distribution are relatively constant and characteristic for species occurring in both localities and the work that has been and is being done has yielded much new knowledge of fundamental interest to all students of insect behaviour. It is becoming increasingly apparent that each species is fitted with the greatest precision into the plant animal complex which forms its environment. It is only by understanding the minutiae of behaviour that we can hope to learn how to control the disease by breaking the link between monkey and mosquito or mosquito and man, or at least to predict where and under what conditions outbreaks may occur as a result of changes in the mosquito population. Finally there is the question of distribution, and this, too, has a special interest since the distribution of the mosquito is closely linked with that of the disease. *Aedes africanus* is a mosquito of the tropical rain forest. The temperate rain forest, the dry forest and the Savanna have each their characteristic mosquito fauna. *Aedes africanus* occurs in none of them, yet in each yellow fever is known

DISCUSSION

to occur We have much to learn regarding the limiting factors by which these regions are defined and the characteristics of the mosquitoes which inhabit them When Edwards' classical work on the Ethiopian mosquitoes was published in 1941, only one rare species of *Stegomyia* had ever been recorded from Northern Rhodesia

Professor B Maegraith Dr FINDLAY has mentioned the small epidemic of yellow fever that broke out in Sierra Leone in 1941 and 1942 I did the autopsy on the first victim and made the tentative diagnosis, which was confirmed by Dr FINDLAY shortly afterwards When we came to look into the history of the Polish officers who died, we found they had perfectly good yellow fever certificates intimating that they had been inoculated with Burroughs Wellcome vaccine in England shortly before arrival in West Africa, and were thus presumably immune A clinical account of these cases appeared some time later in the TRANSACTIONS—I cannot recall the author's name, I think it was one of the medical officers attached to the general hospital in which the patients were treated In this account the disease was described as occurring in inoculated (and, presumably, protected) individuals In fact, and I think Dr FINDLAY will agree with me here, when we looked into the question we found that at least one of the victims had a habit of collecting medical certificates without necessarily having the injections As far as we could ascertain, this man and possibly one of the other two, was probably never injected with vaccine at all Inoculation with the vaccine for protection is a necessary thing, it will produce immunity But it is sometimes dangerous to rely entirely on a certificate as evidence of inoculation I think it is more than likely that the Freetown cases in 1942 occurred in unprotected individuals

Dr P C C Garnham From an adjoining territory, I watched with admiration the work at Bwamba and was fortunate enough to see some of it in progress In Kenya, two forests have been incriminated as foci of the sylvan disease One is just outside Nairobi, the other is on the coast near the ruined city of Gede Searches over several years failed to reveal the presence of *Aedes africanus* in either of these two forests, where it is possible that the vectors are *Aedes deboeri* and *A. adersi* respectively

I believe Dr MAHAFFY originally thought that yellow fever in the monkey was not the whole story in the African forests the monkeys would soon all become immunized and the disease would come to a standstill What is his view today on this point? A second question is regarding the control of jungle yellow fever in Africa Apart from vaccinating the local population, would it be possible to attain control by either (a) killing all the monkeys, or (b) attacking *Aedes africanus* through its breeding places which in some ranges forests are practically confined to pools in the ground buttresses of certain kinds of trees?

Dr C J Hackett I would like to ask Dr MAHAFY why it is that in African jungle yellow fever deaths appear to be exceptional apart from the Nuban Ranges epidemic, whereas in American jungle yellow fever the mortality is more noted? I would also like to hear his opinion regarding the significance of protection tests in mammals other than monkeys reported in the early part of the 1930's. Finally has he any views regarding the origin of the virus of yellow fever in Africa and South America? In other words, what is the present view regarding the "home of yellow fever" which was at one time thought to be West Africa?

Dr Wilson Raa I desire to express one word of caution, and consider it should be said here that it may share the wide publicity this address will undoubtedly have. The history of yellow fever—to any one who has to do with outbreaks of the disease—has two phases. The first during the outbreak is one of extreme fear and perturbation, but this is followed when the emergency ceases, by an unwarranted feeling of complacency. We can never afford this complacent attitude and now less than ever.

In the days before protection was possible we were made aware in West Africa of outbreaks as the result of acute illness among the European and Syrian sections of the community. These afforded the danger signals. Now when these groups are protected by inoculation, the warning signals are not seen, and yet outbreaks occur and will continue to occur. A recent survey in Lagos, where no outbreak had been notified for a considerable number of years, has shown a high rate of immunity among the younger age groups.

In the international field it has been suggested that different steps be taken where the disease is known to occur in clinical form, and in those areas where this is not seen. The danger to the second group is probably greater than in the first. Yellow fever outbreaks are taking place now without our knowledge and any attitude of complacency may result at some date not too far distant of an epidemic of explosive violence.

Dr M T Morgan I can claim but little qualification to take part in the debate except a minor one that it was I who shot down the first monkey in the Matto Grosso Brazil, the blood of which was found to protect. The monkey was a female and had a baby monkey about 2 months old. That baby monkey also gave a protection test. I was interested to hear from Dr MAHAFY that approximately 60 per cent. of the monkeys were found to be protected. I want to know whether he is aware of any mortality among the wild monkeys in that area from yellow fever or is it that they receive an inoculation at an age when they get a mild attack, or even no obvious attack at all—and consequently develop an immunity of any early age, which perhaps increases with further biting? I have a third question. Is there any evidence of migration of these monkeys from the area?

Dr Mahaffy (in reply) I should like, in the first place, to thank all those who have contributed to this interesting discussion

Dr FINDLAY called attention to the fact that immunity to yellow fever has been found in monkeys over a very wide area in Africa. He also cited several instances in which the human disease was observed in West Africa under conditions which suggested that the insect vector was not *Aedes aegypti*. There is a lot of evidence which suggests that the forest type of the disease exists in West Africa and that monkeys play an important role as vertebrate hosts. The forest vector, however, has not yet been found in that region.

Mr MATTINGLY has asked how far the work in Bwamba can be expected to apply in other parts of Africa. He refers, I assume, particularly to the forest vector. The work in Bwamba has provided evidence that *Aedes africanus* is the forest vector of first importance in that small area, and nothing more. It would, I think, be quite unjustified to apply this finding to other parts of Africa without further study. Because of its wide distribution in the African forests, *Aedes africanus* might be regarded as "suspect" in other areas. All one can say is that the work in Bwamba suggests a method which might be used to incriminate the vector in other regions in Africa.

Dr GARNHAM has suggested that, at one time, it was felt that there was probably not an adequate number of monkeys to maintain the cycle in the Semliki Forest indefinitely. It is true that this point was much discussed some years ago. However, evidence now exists which indicates that the infection is, in fact, maintained indefinitely in these animals, and quite independently of any human infection. In this connection it should be pointed out that it is now known that the vector, *Aedes africanus*, is not only present in the forest throughout the year, in the adult state, but that it can survive for long periods even under the most unfavourable conditions. Although the monkey is infective for only a limited period, the mosquito can continue to transmit the infection for several months.

As regards the control of jungle yellow fever, I regard it as extremely unlikely that the control of either the animal host or of the forest vector could be undertaken with any reasonable hope of success. Persons exposed to the sylvan type of the disease can, however, be protected against it by vaccination.

Dr HACKETT has mentioned the apparent low mortality in jungle yellow fever. There is very little exact information available on the mortality due to this type of the disease in Africa. However, the mortality in Africans is low in the urban type of disease and I know of no evidence which suggests that there is any significant difference between the mortality rates in urban as compared with forest yellow fever in Africa.

Immunity to yellow fever has been found in animals other than monkeys in Central Africa. The answer to this question will shortly be available in several papers which have recently been submitted for publication.

Dr HACKETT has asked about the relationship between American and

African strains of virus. I assume he refers to the place of origin of the infection. Before the advent of jungle yellow fever it was the opinion of many observers that the available evidence pointed to Africa as the home of the infection. However if as is now believed, the jungle type of the disease is the original epidemiological type, it becomes impossible to say where the infection originated.

The point raised by Dr WILSON RAE is one of very great importance and one which should be kept constantly in mind, particularly by those responsible for the measures which should be taken to prevent spread of the disease.

Dr MORRAN has asked if there is evidence of mortality in the wild monkeys in Bwamba. As far as I know no African monkey is known to have died of naturally acquired yellow fever infection in Bwamba. If there is any mortality due to yellow fever infection in these animals, it must be very low.

As regards migration of monkeys in Bwamba, observations which have been made by Dr A. J. HADDOW over a period of years indicate that they travel only very short distances. In this connection it is of interest that CAULLEY and his colleagues have recently reported that the normal movements of certain Brazilian monkeys which they have investigated do not extend beyond a few hundred yards.

COMMUNICATIONS

A NEW HUMAN CESTODE INFECTION IN KENYA

Inermicapsifer arcanthidis, A PARASITE OF RATS

BY

H A BAYLIS, M A , D SC ,

Department of Zoology, British Museum (Natural History)

A boy aged 2, the child of white parents living in Nairobi, Kenya, was brought home to England towards the end of April, 1948. Some weeks later (about 13th June) he was found to be passing tapeworm segments of an unusual type. Some of these were submitted by his medical attendant, Dr W A KENRICK, to Dr E R. JONES, Pathologist of the Public Health Département, Maidstone, who reported that they "appeared to be mature proglottids of the cestode *Inermicapsifer cubensis*". At this point Sir PHILIP MANSON-BAHR was consulted, and he took the boy into hospital, where he prescribed filix mas min 15, which, as the patient was somewhat "difficult," was administered the same evening in jam and honey, and was retained during the night. Early in the morning it was followed by syrup of figs drachm 1, after which two complete worms, apparently dead, were evacuated with the stool.

The specimens, which were first preserved in warm 70 per cent alcohol and then transferred to 2 per cent formol-saline, were submitted by Sir PHILIP MANSON-BAHR to Professor J J C BUCKLEY, who kindly passed them on later to the writer for examination and determination. One specimen was

stained with haematoxylin and mounted in Canada balsam by Professor BUCKLEY. Its scolex had unfortunately been lost, probably during collection, but the worm appeared otherwise to be complete. The other specimen, received in fluid although broken into a number of pieces during transit, was quite complete, and provided satisfactory preparations of the scolex and of selected portions of the strobila. It is not of course, to be expected that worms removed by anthelmintic treatment will be found in perfect condition for detailed anatomical study. Some degree of maceration is inevitable, but in the present case the condition of the material was surprisingly good in view of the time that had elapsed between the administration of the drug and the recovery of the worms.

Study of the anatomy of the worms showed beyond doubt that they belonged to the genus *Iuermicapser*. It should, however be pointed out at once that the gravid segments of this genus are so similar to those of some species of *Railletina* that a diagnosis based on gravid segments alone would be impracticable. Apart from *I. cubensis* (Kouri 1939), a species recorded in a number of cases as a human parasite in Cuba, the genus *Iuermicapser* is at present known only in Africa. At least nine species of the genus occur in Hyracoidea (genera *Procavia* and *Heterohyrax*) and four others have been described from rodents. The characters of most of these species are summarized, and their synonymies given, by BAER (1927), but *I. longanythae* Baer 1933, and *I. leporis* Ortlepp 1933, have been described since the publication of his monograph.

It seemed natural to inquire whether the present case might represent an accidental or occasional human infection with one of the species that normally occur in wild African animals. There can, in the writer's opinion, be no doubt that such is the case, the species in question being *Iuermicapser arcanthidis* (Kofend, 1917). This worm has been recorded from a number of species of rats, of which a list is given below (Table II). The collection of the British Museum (Natural History) contains specimens previously determined by the writer as *I. arcanthidis* from *Mastomys comoki* subsp. and *Heliophobus argenteocinctus emoni* with which the present human specimens appear to agree in all essential respects.†

The most complete description of *I. arcanthidis* in the literature seems to be still that of KOFEND (1921), but some details have been added by MEGGITT and SUBRAMANIAN (1927) and by BAER (1927). There seems to be room,

One doubtful species of the genus *I. stidis* Meggitt, 1927 is recorded from bird (Macqueen bustard, *Himantopus macquarii*) while BOUTHWELL and LAKE (1939) have recorded *I. grahami* (Graham) from bird (*Centropus superciliosus leander*). Possibly both these were cases of pseudoparasitism.

† Mr J. R. HUDSON M.S.C.V.S., informs me that *Arctanthidis allynsmithi murekai* is one of the commonest rats around Nairobi. He has (Hudson 1934) recorded *Iuermicapser grahami* (Graham, 1906) from this host. Of the specimen which he has kindly allowed me to examine it seemed to be *I. grahami* and others appeared to belong to different species which was not *I. arcanthidis*.

H. A. BAYLIS

however, for some further description, especially as the species is new to medical literature, and as the present study of it has brought to light at least one anatomical feature that has not hitherto been mentioned

REDESCRIPTION OF *Inermicapsifer arvicanthidis* (KOFEND, 1917)

The following description is based mainly on the two human specimens, but on some points reference is made also to specimens from *Heliofobius argenteocnereus emini* from Tanganyika Territory

SIR PHILIP MANSON-BAHR mentioned in conversation that he had measured the two human specimens, when complete, in the fresh condition, and that each was 25 cm in length. The writer measured them again after preservation, and found the total length of the specimen that had been mounted in several lengths in balsam to be about 17.9 cm, while the sum of the fragments of the other specimen amounted to 18.5 cm. It is, of course, natural that considerable contraction should occur on fixation. The maximum width of each worm, after preservation, was about 3 mm. The number of segments in the two strobilae was about 315 and 360 respectively. In a strobila about 8.7 cm long (without a scolex) from *Heliofobius*, the number of segments was about 235. There is an unsegmented "neck" of variable length behind the scolex. The segments are broader than long throughout the strobila, except for the last few gravid segments, which may be slightly longer than broad.

The single scolex available from the human case measures (in Canada balsam) about 0.45 mm in width, or 0.52 mm in a diagonal direction—i.e., at the widest part as it lies in the preparation (Fig. 1). The scolices of two specimens from *Heliofobius* measured 0.54 and 0.55 mm in width. There is, of course, no trace of hooks or a rostellum, the unarmed condition being characteristic of the genus. The suckers are relatively large and highly muscular. In general shape they are subglobular, with deep cup-like cavities. Their greatest outside diameter is 0.19 mm in the human specimen, and almost exactly the same in specimens from *Heliofobius*. The scolex and suckers are slightly smaller in material from *Mastomys*.

With rare exceptions, the genital pores are unilateral. They are situated on the left side of the segment. Their exact position, however, naturally varies with the degree of extension or contraction of the segment. The male and female ducts pass between the dorsal and ventral excretory canals, and dorsally to the longitudinal nerve, of the poral side. The scolex and suckers are situated on the left side of the segment. Their exact position, however, naturally varies with the degree of extension or contraction of the segment. The male and female ducts pass between the dorsal and ventral excretory canals, and dorsally to the longitudinal nerve, of the poral side.

There is a highly developed layer of subcuticular gland-cells (Fig. 3, sc) which, in whole preparations, tends to make the specimens opaque and renders the internal organs rather difficult to observe. The main longitudinal musculature, which lies below this glandular layer, is not very highly developed, and consists of bundles of fibres which are irregular in size and arrangement. There appears to be no regular layer of transverse musculature dividing the parenchyme

into cortical and medullary regions, but there are numerous dorso-ventral fibres passing right through the central region.

The excretory system is of special interest. The usual pair of simple longitudinal canals is present dorsally but the ventral system, instead of the single pair of longitudinal canals, with transverse commissures at the junctions of the segments, typical of the majority of cestodes, consists of a number of longitudinal vessels (Figs. 2 and 3, *v*) which appears to vary from four to six in different strobilae and in different portions of the same strobila. These main vessels are connected at frequent but irregular intervals by narrow and branching transverse vessels, forming a sort of network. The presence of an excretory system of this type, which is visible in favourable and suitably stained whole preparations, has been confirmed by means of serial sections both of the human material and of a specimen from *Hebephobus*. It cannot yet be stated whether this type of excretory system is a constant generic character in *Iernicapsifer* but it is known to occur in some other species, e.g. *I. hyrcani* (Rud. 1808) and *I. guineensis* (Graham, 1908) [synonyms, *Thynnosoma gambianum* Beddard, 1911 *Zschokkerella macrole* Baylis, 1915]—see BAYLIS (1915). Multiplication and branching of the ventral canals has been described in species of several other genera of Anoplocephalidae (*Anoplocephala*, *Cittotarsus*, *Schisto-tarsus*, *Multicapsiferus*, *Oochoristica* and *Progamotermis*), and also occurs in certain Davaineidae.

The cirrus-sac is relatively small and oval in shape. It measures about 0.1 to 0.12 mm. in length and 0.03 mm. in maximum width in the human material, and the same dimensions were found in specimens from *Hebephobus*. It should be noted that KOFKOV (1921), in his description of the original material of *I. arcticus*, gives the dimensions of the cirrus-sac as 0.14 to 0.15 mm. \times 0.052 mm. The writer finds that a length of about 0.15 mm. can be obtained if the measurement is taken from the actual opening of the genital pore—i.e. if it includes the slight genital atrium into which both cirrus-sac and vagina open. The inner end of the cirrus-sac just reaches the lateral nerve of that side. There

LEGENDS TO FIGURES

FIG. 1.—*Iernicapsifer arcticus* (human case). Scolex.

FIG. 2.—*Iernicapsifer arcticus* (human case). Dorsal view of young mature segment (semi-diagrammatic). *c.s.* cirrus-sac *d* dorsal excretory vessel; *n.*, longitudinal nerves *ov* ovary *t* testes *v* ventral excretory vessels *v.a.*, vagina; *d.v.* vas deferens *vit* vitelline gland.

FIG. 3.—*Iernicapsifer arcticus* (specimen from *Hebephobus argenteocinctus*). Transverse section through young mature segment, cuticle *d* dorsal excretory vessels; *d.m.* dorso-ventral muscles *l.m.* longitudinal muscles *n.*, *n.*, longitudinal nerves *sc* subcuticular layer *t* testes *v* ventral excretory vessels (two of these on the right of the figure showing branches) *v.a.* vagina *d.v.* vas deferens.

FIG. 4.—*Iernicapsifer arcticus* (human case). Gravid segment, showing egg-capsules.

FIG. 5.—*Iernicapsifer arcticus* (human case). A single egg-capsule showing 12 eggs.

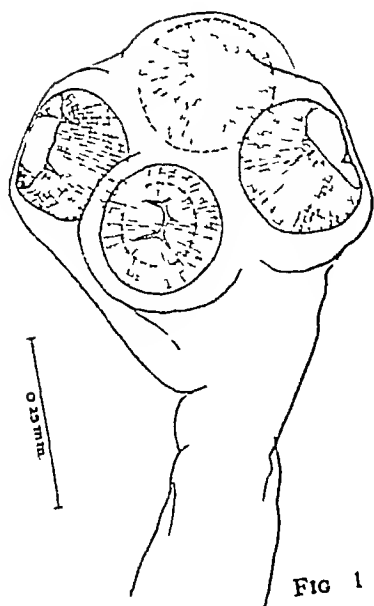


FIG 1

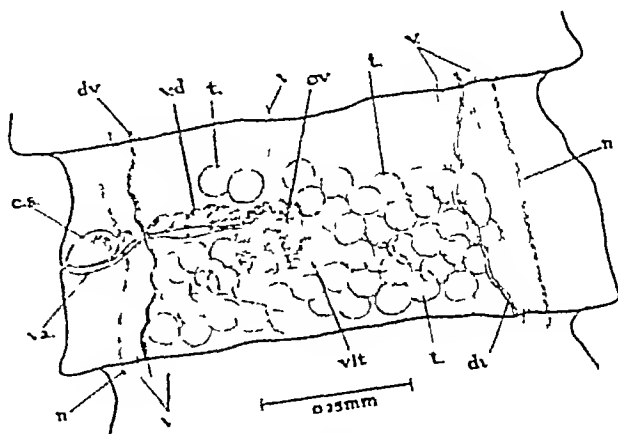


FIG 2

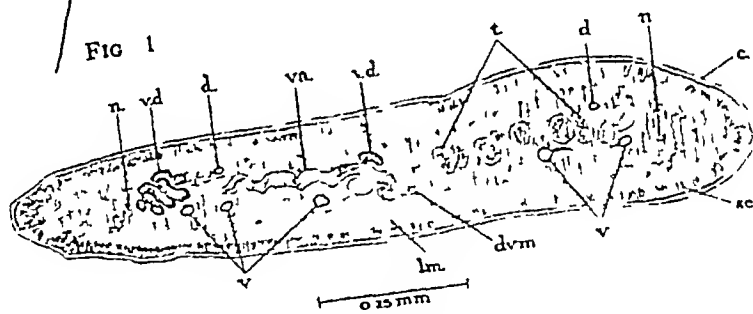


FIG 3

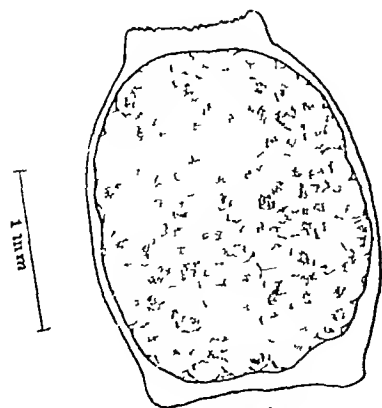


FIG 4

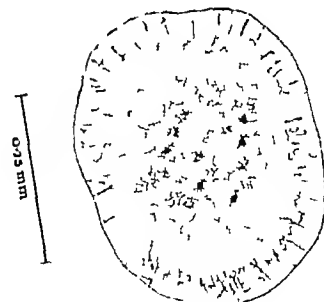


FIG 5

H A BAYLIS

appears to be no specialized internal or external seminal vesicle, but the vas deferens forms a mass of coils which provide ample storage for sperm.

The vagina opens into the genital atrium, immediately behind the cirrus-sac, and runs directly inwards behind that organ and the vas deferens to the female gland-complex, which lies somewhat on the poral side of the middle line of the segment. In its distal portion it has a narrow lumen and an external coat of gland-cells. In the proximal half it becomes thin-walled and capable of expansion into a fusiform swelling which seems to be the only seminal receptacle.

There appear to be from 48 to 55 testes in each mature segment, of which the greater number (31 to 38) are on the aporal side of the female glands, and a smaller group of 16 or 17 on the poral side. Their size is variable, but when fully developed the largest attain a diameter of about 0.06 mm. As they increase in size they occupy almost the whole of the medullary region, extending laterally beyond the excretory canals and as far as the lateral nerves. The poral and aporal groups become confluent behind the female glands, while a few testes of the aporal group come to lie in front of the ovary or even on the poral side of it.

The ovary consists of a fan-shaped mass of lobes which, when fully developed, measures 0.2 mm or more in width. Behind it, and nearer to the middle of the segment, lies the compact vitelline gland, which is more or less kidney-shaped and has a maximum width of about 0.1 mm. The uterus apparently develops very rapidly as a branching or reticular structure extending over the greater part of the segment. It is visible only in one or two segments at a time, and equally rapidly disappears, becoming transformed into egg-capsules. The number of these formed in each segment (Fig. 4) seems to be very variable. In the human specimens the writer found about 80 to 125, and in specimens from *Helophobius* about 42 to 70. KOFEND gives their number in the original specimens of *I. arvicanthidis* as 40 to 50. In all probability the number varies with the size of the specimen and of the segments. The capsules (Fig. 5) are composed of masses of thickened parenchyme, the central portion being denser than the outer layer, which shows a number of radiating fibres. In the earlier stages of their formation the capsules are so crowded together as to assume polyhedral shapes, but later the space in which they lie seems to enlarge so as to allow them to expand, and they then assume a more or less ovoid form, with a greater diameter of 0.3 to 0.6 mm, the average being about 0.4 mm. In the material from *Helophobius* they are rather smaller (0.25 to 0.35 mm).

Each capsule contains about 12 to 15 eggs in the human material. In specimens from *Helophobius* the number is also about a dozen, while KOFEND gives 11 to 13. The onchospheres are somewhat ovoid and very small, having a greater diameter of 12 to 16 μ . They are enclosed in vitelline membranes which, in most preparations, are collapsed and crumpled. The embryonic hooks are about 6 μ long.

appears to be no specialized internal or external seminal vesicle, but the vas deferens forms a mass of coils which provide ample storage for sperm

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the intermediate hosts not only of *Cottolasmus* but of several other genera (*Anoplocephala*, *Paramoplocephala*, *Nomemnia*, *Bertiella*, *Thysanura* and *Catenotarsus*). Now it seems worth while to point out that the eggs of typical Anoplocephalidae are very small, and are stored in large numbers, but each separate from its neighbours, in a sac like or branching uterus. They are not grouped, several together in egg-capsules, and frequently though not invariably

TABLE II
RODENT HOSTS OF *Isomacropus arviculoides*.

Host	Locality	Recorded by
Family MURIDAE.		
Subfamily MURIDAE.		
<i>Arviculles niloticus</i> [tanzanianus]	Sudan	Kofoid, 1917
<i>kordofanicus</i> Wettersin		
<i>Arviculles niloticus rufinus</i> (Temminck)	Dahomey	Joyeux & Baer 1927
[<i>Arviculles</i> sp.]		
<i>Hybomys nicholsoni</i> (Peters)	Nigeria	Joyeux & Baer 1930
<i>Lemnicomys tristis</i> (Linn.)	Nigeria	Joyeux & Baer 1930
? <i>Oenomys hypomelanus</i> (Pucheran)	Dahomey	Joyeux & Baer 1927
[<i>Mus rufinus</i>]		
<i>Pelomys (Galeomys) campae</i> (Huet)	French Guinea	Joyeux & Baer 1927
<i>Rattus (Mastomys) coucha erythrolaemus</i> (Temminck)	Nigeria	Joyeux & Baer 1930
[<i>Mastomys erythrolaemus</i>]		
<i>Rattus (Mastomys) coucha micradon</i> (Peters)	Tanganyika	Baylis, 1934
<i>Rattus (Mastomys) coucha</i> (subsp. ?)	Belgian Congo	Baylis, 1933,
<i>Thalassomys (Mus) maggi</i> (Roberts)	S. Africa	Baer 1925.
Subfamily DIPSOSAURIDAE.		
<i>Stenomys praecox</i> Peters	S. Rhodesia	Baer 1933
Subfamily GERBILINAE.		
<i>Tatera kropsi</i> Wroughton	Nigeria	Joyeux & Baer 1930
Subfamily OTOMYINAE.		
<i>Otomys irroratus</i> (Brents)	S. Africa	Baer 1925.
Family B. TITIDAE.		
<i>Heliophobius argentocroceus croceus</i> Nock	Tanganyika	Baylis, 1934
[<i>H. ericki</i>]		

Mus rufinus might refer to *Arviculles niloticus rufinus* but in view of the locality VI Hayman thinks it more likely to have been *O. hypomelanus*.

the inner shell of the egg, or embryophore, is of the type known as a "pyn form organ," being drawn out on one side into a process which may form two long filaments. The diameter of the outer shell or membrane of the eggs, in these genera, is not more and often considerably less, than 85μ , and they are thus of a size which can evidently be swallowed by mites.

The eggs of *Isomacropus* on the other hand, are enclosed, in clumps of varying number in fairly tough capsules which, as we have seen, in *I. arviculoides* have an average diameter of about 400μ . It is suggested that,

as these capsules might be rather large to be swallowed by mites, it is more likely that the intermediate host will prove to be a somewhat larger creature—perhaps an insect. Here it is instructive to compare *Inermicapsifer* with another genus, *Raillietina*, in the typical subgenus of which egg-capsules of an almost identical kind are produced. *Raillietina*, which not only possesses a rostellum armed with numerous minute hooks, but in which the suckers are also armed with rows of hooks or spines, is placed in the family Davaineidae. It is a very curious fact that a species of *Raillietina*—*R (R) baeri* Meggitt and Subramanian, 1927—occurs in some of the same African rats as *Inermicapsifer arvicanthidis*, and shows, except in having an armed scolex and an excretory system of a different type, so close a resemblance to that species as to be by no means easy to distinguish from it. The writer has, in fact, found a preparation (without a scolex) of *I arvicanthidis* among specimens previously determined by himself as *R baeri*. Hence, until examination of the scolex placed the matter beyond doubt, it was possible that the human specimens might have been misdetermined as *R baeri*.

Certain species of *Raillietina* occurring in poultry (*R (R) tetragona*, *R (R) cesticillus*) are known to make use of beetles and ants as intermediate hosts, and it may be suggested, as a possibility for future investigation, that the intermediate hosts of *Inermicapsifer* may prove to be small insects rather than mites. GRADWOHL and KOURI (1948) state that over 75 cases of human infection with *I cubensis* have now been reported in Cuba. Most of the patients seem to have belonged to the white race, and the great majority of them have been children. This last point may be of considerable importance, since the habits of children seem rather more likely than those of adults to lead to the ingestion of possible intermediate hosts. It is noticeable, also, that a large proportion of the cases of human infection with certain species of *Raillietina* (*R (R) celebensis*, *R (R) demerariensis*, *R (R) formosana* and *R (R) madagascariensis*) have been in children. In this connection it may be of interest to quote one or two sentences from the notes supplied to Sir PHILIP MANSON-BAHR by the mother of the young patient from Nairobi. After stating that he had never eaten flesh, except chicken or well-cooked scraped liver, she wrote "his diet was mainly pawpaw, orange, apples and tomatoes uncooked, also cakes and bread made locally, and imported breakfast cereals. From time to time we took picnic meals on to the plains around Nairobi, and it is possible that N—— could have put something in his mouth from the ground or sucked dirty fingers."

This is, perhaps, not the place for the discussion of a purely taxonomic question, but it may be briefly mentioned that the points of similarity already noticed between *Inermicapsifer* and *Raillietina* raise some doubts concerning the validity of the classification at present accepted, and suggest that *Inermicapsifer* may possibly be more closely related to the Davaineidae than to the

Whilst a close study of 1,500 slides of sheathed microfilaria were being made in 1937-38, about 12 examples of *W bancrofti* were found, and confirmed by LEPPER (1938). These microfilariae have not been found in any case of elephantiasis. So far there is no evidence that elephantiasis hydrocele, lymphocele, or lymphadenitis cases are caused by *W bancrofti*.

Loa loa occurs in latitude 4° to 6° N—from French Equatorial Africa in the west to a line just west of longitude 30° E., and South—well into the Belgian Congo. It has not been reported east of longitude 30° E., or authenticated in Uganda. In this area upwards of 20 per cent. of the indigenous population are infected and, 15 years ago about the same percentage of British and Syrian officials. Its only proved vector is *Chrysops distinctipennis* and *C longicornis*, which occur in the ratio 26:1. The development of *L. loa* embryos to maturity in these chrysops were described in detail in the above-mentioned paper (WOODMAN and BOKHARI, 1941). Development was much slower than that in *C. nleae* and *C. dimidiata*, as described by CONNALL and CONNALL (1922).

The following table summarizes the comparison

COMPARATIVE DEVELOPMENT OF *LOA LOA* EMBRYOS IN CONNALL AND CONNALL'S, AND S. SUDAN EXPERIMENTS.

Stage.	Conn. & Connall, Nigeria.	S. Sudan
1 hour	16-250 μ 10-1	
2nd day	210 μ 23 μ	190 μ 10 : 211-216 14 μ
3rd	290-300 23 30 μ	
4th	290 25 μ Variable	
5th	0.8-1.0 mm. \times 37 μ } Maximum	
6th	1.0 mm. 49 μ } changes	
7th	1.3 mm. 40 μ	250 14.0 μ
8th	1.7 mm. 30	30-373 18-23 μ
9th	1.8 mm. 27 μ	
10th	2.0 mm. 3 μ . Completed, flukes did not survive longer	Maximum changes 373-690
11th		
12th		800-990 μ 2
12th-18th day	Irregular	1.04-1.84 mm. 20-22
18th-24th		1.04-2.0 mm. 20-30 μ

HUGH M. WOODMAN

Although CONNALL and CONNALL did not include data on the temperature and humidity when they did their West African experiments, it is believed that the Southern Sudan conditions were not vitally different. Dissections were carried out in the Sudan in the dry season and during the rains, when the temperature was from 85° to 90° F by day, and with a humidity up to well over 90 per cent.

BASU and RAO (1939) published a paper on the effect of humidity and temperature on the development of *mf banerofti* in *Culex fatigans*, and showed that they reached maturity in 9 days at a temperature of 90° F, 10 days, at a temperature of 80° F, 20 days, at a temperature of 70° F, and 47 days, at a temperature of 60° F, with a humidity of 80 to 100 per cent. (He noted, incidentally, that the age of the patient seemed important. Up to the age of 50, 26 per cent of the mosquitoes developed the embryo. Over the age of 50 80 per cent.)

Although this referred to a different species of filaria, it would appear improbable that climatic factors can explain the slowness of development as compared with that in *Chrysops* in West Africa, as it is only in temperatures below 80° F that a big difference shows itself in length of time of development.

These experiments suggested that while *L. loa* could and did develop in these species of *Chrysops* (of 600 flies dissected in the wild state 0.6 per cent were found positive for embryos of *L. loa*), it is possibly not the optimum, or even the commonest, vector of the parasite in this part of Africa, for the following reasons: (a) The furtive habits of the fly and its reluctance to bite man in the wild state. (b) The rarity with which it is found in or around houses. (c) The difficulty in finding it, except on cattle, in the hotter hours of the day. (d) The irregularity of development and the considerable proportion of embryos of *L. loa* that become immobile or cretified, instead of progressing to maturity. The latter resemble those found in *Haematopota*—an unnatural host in which only partial development can occur. (e) Subjects can become infected with *L. loa* without ever having seen, or having had conscious contact with, *Chrysops*.

In the search for an alternative vector, 40 specimens each of *Haematopota*, *Stomoxys* and *Glossina* were fed on infected volunteers and dissected. The results were negative except in the case of *Haematopota*, which showed development up to the equivalent of the third day in *Chrysops* (in CONNALL and CONNALL's experiments). The only remaining common tabanid in the area known to be a voracious feeder on man, and which has not yet been experimented with, is *Hippocentrum trimaculatum* (News), and *H. versicolor* (Aust).

The adult embryo of *L. loa* is a good deal longer than that of *W. bancrofti*, being 2 mm × 0.02 mm, compared with 1.4 mm × approximately 0.02 mm, although the latter can be carried by mosquitoes as small as *Anopheles funestus*. There is plenty of evidence that a very large filaria can develop in a very small insect even if the passageway is smaller in places than the breadth of the parasite. In this connection FENG's (1936) work on the behaviour of *mf malayi* is of great interest. He showed that not only could *A. hyrcanus*, var *sinensis* (the vector in China) hold as many as 25 fully developed embryos in its labium, but that these could disperse into all parts of the insect, including the legs and basal joints of the antennae. These gymnastics are made possible by the fact that the worm can undergo stricture to half its normal diameter, which it often does as a natural procedure. He observed, moreover, that the worm can pass

out of the tip of the labella, and this was photographed. Similar work has been done by YAMADA and IYENGAR (1928 and 1932).

SHARP's demonstration (1928) of the development of the large form of *A. persians* in *Callicoides aspersus* is a most remarkable example of disproportion. He estimated the embryo to grow 0.75 mm. to 1.0 mm. in length while its insect vector is itself only 2 mm. in total length, and yet was capable of delivering six mature embryos from its labium at one time.

Next to the possibility of *Hippocentrus*, it is suggested that there may be a mosquito vector of *L. loa*. It is known that the same blood nematode can be carried by arthropods of widely different kinds. Examples of this are *W. bancrofti* developing in *Phlebotomus chinensis*, and *P. sergenti mongoleus* (IAO, WU and SUN, 1938). These were found infected in the wild state, while other experimentally fed flies showed degrees of development which might have been completed had they lived. *Dirofilaria immitis* can develop in dog fleas as well as in *Culex Aides*, and *Anopheles* (BREINL, 1921 and HEGNER *et al.*, 1938). *A. persians* can develop in *Ornithodoros moubata* (NUTTALL, 1916).

L. loa has been known to infect a European after 5 months exposure in the endemic area although no known contact with chrysops had ever been made. In this case the adult was seen crossing the eye within a year calabar swellings were frequent, and eosinophilia reached 70 per cent. in the same period.

During 9 years observations no microfilariae were seen. WOODMAN and BOXHALL's paper above referred to drew attention to a possible connection between the earliest state of varicolympheocele, and of inguinal hernia, with *L. loa* infection.

Acanthocheilomena persians occurs in over 50 per cent. of adults, although it has not been reported in Europeans. It occurs throughout both areas marked on the map but is commonest on the south-western border. Most authorities seem agreed that it is a harmless parasite. Although such evils have been laid at its door as oedema and calabar swellings (BRAUN and SEIFERT 1926), non-gonococcal urethral discharge (SHARP 1928), epigastric pain and hepatitis (GARRETT E., 1945), and filarial fever (MOLZER, 1939 and SINGH 1928), this is not the generally accepted view and there seems little evidence in support of it in the Southern Sudan.

Filarial fever certainly may occur indeed, it is difficult to believe that a blood swarming with a mass infection of *mf. persians* could fail to cause some malaise or febrile disturbance at times. Nevertheless, bloods do appear to swarm with them in patients admitted to hospitals with other complaints, without any such condition being demonstrable.

Species of *Callicoides* have been collected and sent to D. J. LEWIS, who has had them identified by Dr MACFIZ, of the British Museum, as *Callicoides*

HUGH M. WOODMAN

grahami Aust, *C. milnei* Aust, *C. fulvithorax* Aust The vector for this area is presumed to be *C. grahami*. This has been reported as common in the Belgian Congo at Nepoko, Professor DUBOIS and FORRO (1939) found it there in the skin from the thigh in 31 cases of elephantiasis. SHARP (1924) found 38 to 40 per cent of adults harboured it in the Cameroons. I have not seen reports of it from French Equatorial Africa. It has not been found in the Southern Sudan.

IDENTIFICATION OF BLOOD MICROFILARIAE UNDER FIELD CONDITIONS

The three blood microfilariae with which this paper is concerned—*L. loa*, *W. bancrofti* and *A. persans*—have had their differences in both the live and stained preparations beautifully described and illustrated by SHARP (1924) and other workers.

There is no difficulty with *A. persans*, whose smaller size and rapid movement in the fresh state distinguishes it from the sheathed microfilariae. *W. bancrofti* is not seen often enough to be certain of distinguishing it from *L. loa*, unstained, although its lashing movement, while remaining comparatively stationary, distinguishes it from *L. loa*, whose deliberate snake-like movements propel it in due time across the field.

In specimens stained with methylene blue, haematoxylin, or giemsa, it is noticeable how clear-cut and circumscribed are the nuclei of *W. bancrofti* compared with the larger, deeper-staining, and "hazier" nuclei of *L. loa*. This, and the fact that dilute giemsa stains the sheath of *W. bancrofti* but not the sheath of *L. loa*, that *L. loa* has a pointed whip-like tail which is usually flexed and lies in angular, ungraceful curves, compared with *W. bancrofti*, which has a tapering tail and lies in smooth regular curves, that nuclei extend on nearly into the tip of the tail in *L. loa* but terminate earlier in *W. bancrofti*, leaving a clear space towards the tip, are sufficient guide under field conditions.

Onchocerca volvulus The "Jur" or "Sudan endemic blindness" area lies in the Bahr el Ghazel between longitude 30° E and the frontier of French Equatorial Africa, and between latitude 6° and 9° (Map 1a). This is to the north of the region described in connection with *L. loa*. BRYANT (1935) has given an account of the disease in the former area. The latter region can be described as on the fringe of the hyper-endemic onchocerciasis area. It is a fairly thickly populated region. Blindness due to *O. volvulus* hardly exists save at two points (where it is less than 0.5 per cent), but occasional cases of nodules due to this filaria present at hospital.

As a Government resettlement scheme of these people (the Azande) is in progress, which might bring them within range of infected *Simulium damnosum* breeding places, it was recently decided to carry out a preliminary investigation of *O. volvulus* in this area.

In spite of the large number of hydrocele operations, no microfilaria can usually be demonstrated in tunica vaginalis, fluid, or smears (WOODMAN and BOKHARI, 1941), and their connection with *O. volvulus* seems doubtful. WANSON, HENRAAD and PEEL (1946) investigated 87 cases of hydrocele in the Belgian Congo, and also found them all negative. HAWKING (1939), in an *O. volvulus* area in Kenya, found 10 out of 80 cases positive for that filaria. BRYANT (1935) also found, in the endemic-blindness region that he was describing, many hydroceles positive for *O. volvulus*.

The two most probable sites in the area chosen for this investigation were on the river Suf in whose rapids *Somabru* is known to breed, and near which there are people living. These are, in fact, the only two rapids thus infested of any major importance, and near which fairly big native populations are firmly established.

D. LEWIS confirmed in 1945 that *S. somabru* bred in considerable numbers on *Phragmites* and other stems and vegetation caught in the swirl of the waters of these rapids, although at the time of this survey (February) there was none to be seen. They are definitely seasonal and are believed to be common from April to November. *S. marci* does not occur in the Sudan.

A survey was made by Dr R. KUNK and myself of over 1,400 people of all ages () from whom abnormal skin or eye condition or nodules, leukoids, warts or benign tumours, such as fibromata, were selected and examined (5) from whom control group of persons taken at random and seemingly healthy were selected. The skin in both groups was examined for microfilaria. The two places selected were approximately 100 miles apart on the river Suf and are referred to as Suf I and Suf II (Vizip).

In this survey skin snears were taken at first by shaving off small piece of skin and dropping it into vial of saline centrifuge, and removing the fragment 12 hours later and examining the deposit. It was found, however that shaving off 0.5 to 1 cm. of dermis, and making direct smear on to slide was quicker and much more successful method. It was in fact found that slides were positive where saline deposits were negative. Care was taken to avoid contaminating with blood.

Where nodules were present, the skin was taken from the surface or immediate vicinity of the nodule. Otherwise the sample was taken from any part of the trunk or arm. Warts were particularly searched for and when present were always positive.

Suf I Survey

Sixty-four people were selected from the total population of 800 after general inspection, who were suffering from abnormalities possibly due to filariasis such as nodules, xeroderma or leishmaniasis, and eye complaints. The ages ranged from 12 to 60; there were 20 women and 44 men. 33 had *O. volvulus* in the skin, i.e. nearly 50 per cent. of those selected. Of these 33 10 had nodules (12 per cent. of the total); 20 had skin abnormalities and seven had eye conditions (five being blind or partly blind).

It was an interesting point that six of the specimens of skin taken from nodules were negative.

Suf II Survey

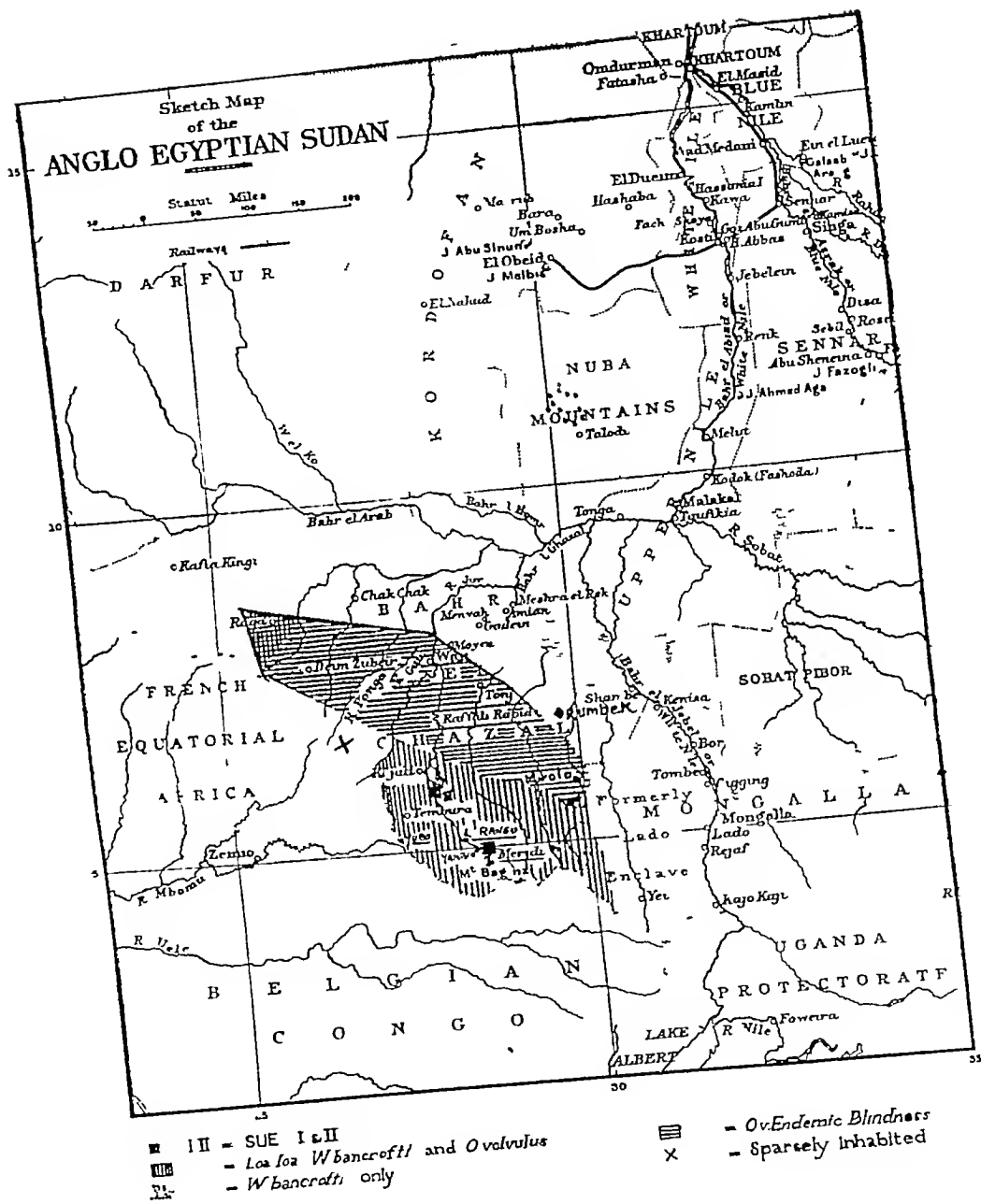
In this survey 40 persons were selected from total population of 840 as showing similar abnormalities. The ages ranged again from 12 to 60. 20 had *O. volvulus* nodules i.e. 50 per cent. of the population. 33 of the 40 had *O. volvulus* microfilaria in the skin, i.e. 83 per cent.

A group of 44 seemingly healthy persons were also selected as controls, and these showed 34 positive for *O. volvulus* in the skin, i.e. 77 per cent.

Only four of the 40 subjects had eye complaints, and two were blind. Only one skin specimen from nodule was negative. It was noticed that the majority of the nodules appeared to be on the ribs rather than on the skin crests.

The chief findings can be summarized as follows

Number examined.	Maximum percentage nodules.	Maximum <i>O.v.</i> in skin of whole population.	Maximum percentage.
1 440	31	per cent.	45



For comparison, figures from a sample group of people living at one of the worst foci of endemic blindness in the Sudan, near Wau, and recently carried out by Dr R. Bray, is of interest.

Number examined.	Maximum nodules Percentage.	Percentage O. in skin.	Blindness.
11	45	91	10 per cent. of adults

A very much higher percentage of positive cutaneous infections in the Sud surveys was thus found than was expected. The percentage of nodules was low and the percentage of eye conditions directly attributable to onchocerciasis very low.

The tribes in parts of West Africa, and the Lumbwa tribe in Kenya definitely associate *Simulium* with nodules and, in some cases, with blindness but the Lumbwa belief is that a person must be exposed incessantly to being bitten before acquiring nodules. This surmise appears to be entirely borne out by scientific observation, and it seems probable that the skin can become considerably infested with the microfilaria before, or during, the formation of nodules and without causing endemic blindness.

Now that VAN DEN BERGHE (1941) has demonstrated that the adult female can distribute her offspring around the connective tissues before becoming encysted, the latter very possibly being the terminal phase of her life history



Chrysops discoloripes.

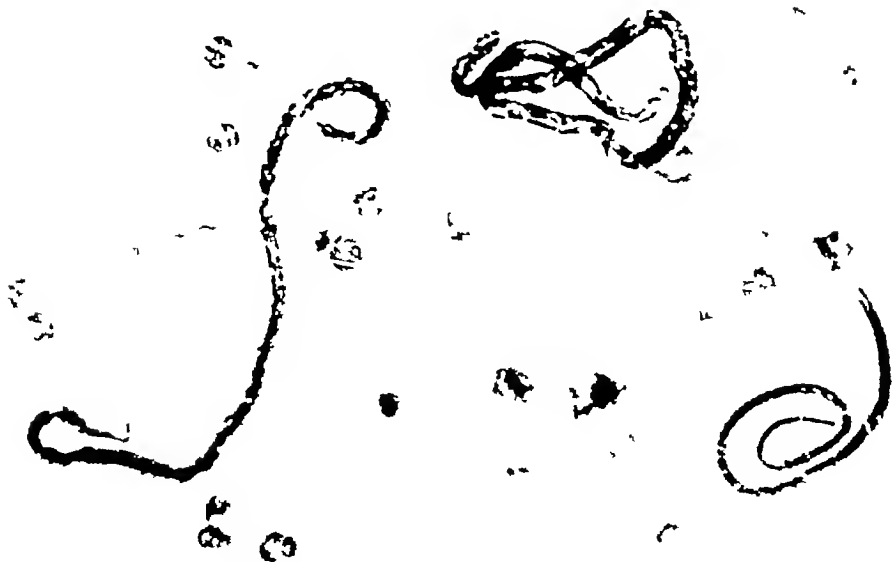


Chrysops longicornis.

PLATE I



Figs 1 and 2 *Loa loa* showing typical flexed tail position



Figs 3 and 4 *Loa loa* and 4 persons

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Chrysops distinctipennis



Chrysops longicornis



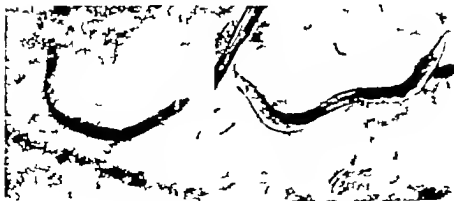
Figs 1 and 2 *Loa loa* showing typical flexed tail position



Figs 3 and 4 *Loa loa* and *L. perstans*

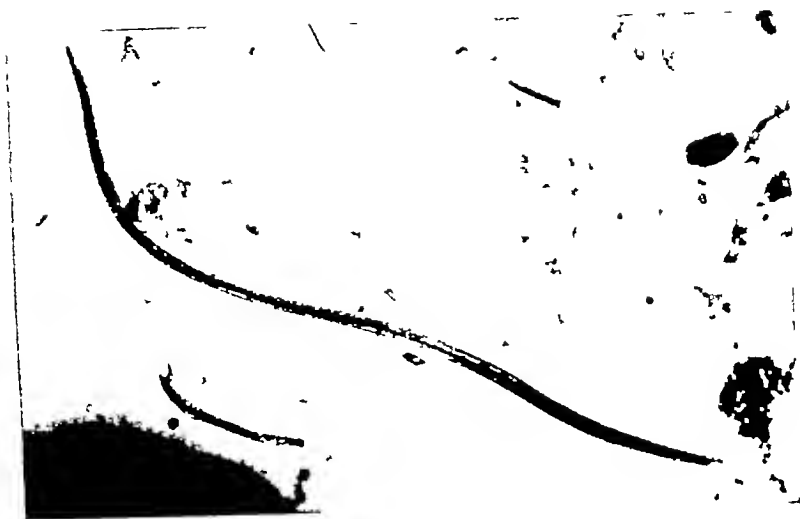


Fig. 1. *Loa loa*. This case showed 33 per field under lower power



Cultured and degenerating *Loa loa* in *Harmatopoda*

PLATE III

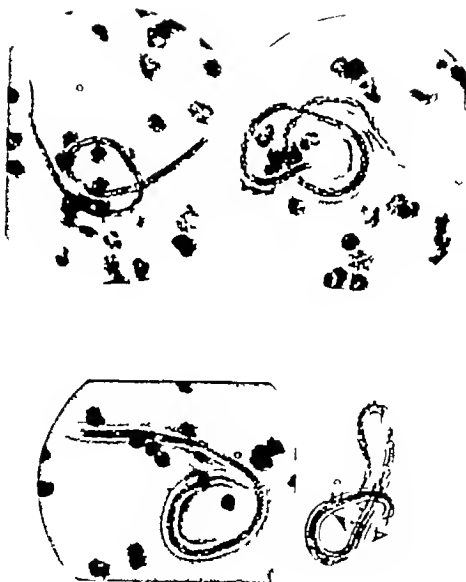


Mature *Loa loa* embryo, 20 days from *Chrysops*



Onchocerca volvulus in skin

PLATE IV



The first slides of infusorians in the Sudan

HUGH M. WOODMAN

the presence of cutaneous infection may not necessarily indicate a heavy infection with adults or future serious complications. A 77 per cent rate of positive skins in a population is, however, more than sufficient warning of the danger of living in close proximity to *S. damnosum* breeding places.

The relationship between *O. volvulus* and the pathological conditions to which it is believed to give rise, vary greatly in neighbouring territories of Africa and for comparison some extracts are given from the Congo and East Africa

Territory	Date	Author	Remarks
Belgian Congo, Leopoldville	1945	WANSON and HENRAAD	Nearly 100 per cent of skins positive in fisherfolk Practically no blindness
Belgian Congo, Leopoldville	1938	HISETTE	50 per cent people have <i>Ov</i> eye con- ditions in hyperendemic areas
N Congo	1941	VAN DEN BURGHE	66.3 per cent. skins positive 61.2 per cent have nodules 12 of 28 blind had larvae in conjunctiva 11 of 26 cases of elephantiasis had micro- filariae
N Congo	1939	DUBOIS	56 per cent elephantiasis cases showed <i>O. volvulus</i>
Kenya	1940	McMAHON and HARRIS	51 per cent. skins positive 31.1 per cent of positive skins had nodules 22.8 per cent. of skins, without mf, had nodules
Kenya	1939	HAWKING	1st series 38 per cent had positive skins Only one had nodule 2nd series 53 cases with nodules showed 62 per cent positive skins
Kenya	1940	HAWKING	Of 80 hydroceles—10 positive for <i>Ov</i>
Kenya (S Kavirondo)	1944	GARNHAM	60 per cent skins positive Incidence of blindness high

The cases of blindness seen, if caused by *Onchocerca*, did not exhibit any of the macroscopic appearances described—punctate keratitis, pyriform or distorted pupil, iritis, etc., but they were beyond the acute state, some of them may have shown characteristic posterior changes as described by HISETTE.

(1932) and BRYANT (1935). Ophthalmoscopic examinations were not done. It can be presumed however that some at least were blind from causes other than *O. colvini*.

Nothing is yet known of the local bionomics of *S. danussoni*. In the neighbouring Uelle district of the Belgian Congo the range is up to 500 yards from the river and 18 per cent. of the fly are infected (VAN DER BEEK, 1941). In the South Kavirondo district of Kenya 8 per cent. of *S. danussoni* are infected and the range is up to 700 yards from water (McMAHON 1940). GARIBOLDI (1933) however, states that in Uganda the fly is found up to 30 miles from its breeding place, and W. J. HEDGECOCK and PIER (1945) have found it 45 miles away around Leopoldville. They admitted that wind over the open river at the latter was a big factor in influencing the range. McMAHON found that a frequented water point in open country 14 miles from an infested stream showed a very low density of *S. danussoni* (the vector in Kenya).

Generally speaking, in the savannah bush forests of the Southern Sudan, wind is not a very important consideration. The nature of the country, the vegetation, humidity and proximity of small mammals, birds and possibly reptiles for the female to feed upon, suggest the unlikelihood of the fly being any serious menace beyond 1-mile range from rapids or water.

Pending closer entomological study it might be assumed that native homesteads are safe from assault by the fly at 1-mile distances from infested rapids. The custom of the people in visiting infested rivers frequently to catch fish and hunt, militates against breaking the man-simulium contact completely and this custom is prevalent among most tribes of Central and East Africa. In the river Sudd country the month of maximum fishing activity is December when, thanks to the seasonal incidence of *S. danussoni*, usually this fly is not found.

PREVENTIVE MEASURES AND CHEMOTHERAPY

The prevention of *L. loa* and *H. bancrofti* infection naturally depends upon the avoidance of their vectors when known. In the case of *O. colvini* it is a question of the avoidance of *S. danussoni* in the Sudan, of *S. watti* in the Congo and East Africa, and possibly also of *S. albertopolatum* (in which partial development has recently been demonstrated by HEDGECOCK *et al* (1944) in the Congo).

Recent experiments with DDT appear to prove that *S. watti* can be completely controlled. An emulsion in the strength of two parts per million of water discharged into a river for 35 minutes has been shown by GARIBOLDI and McMAHON (1947) to be completely successful in destroying the pupae and larvae. Although the eggs, pupae and larvae of this species have not yet been found, it seems almost certain that they occur in rapids and cascades and on vegetation, similar to that which suits *S. danussoni*. Further progress may find a means of control of *S. danussoni*. Unfortunately DDT has the disadvantage of destroying fish.

In 1937 various drugs were tried out on cases of *L. loa*, such as acriflavine by the mouth, on the lines of Fisher's treatment of schistosomiasis in the Congo (1934).

Certain aniline dyes such as methylene blue, trypan blue, and fluorescein and antimony in small doses were employed. Results were all negative except in the case of methylene blue the use of which had been recommended as long

HUGH M. WOODMAN

ago as 1916 (NUTTALL) by AUSTIN FLINT This drug was given in courses intravenously, beginning with 2 c c of 5 per cent solution working up to 5 c c of 5 per cent solution, previous experience having been gained in its use in large-scale treatment of leprosy (WOODMAN, 1937) The racial reaction was slight and seldom gave rise to the toxic symptoms of headache, backache, giddiness, or syncope, sometimes said to be associated with it (The influence of this drug in a 1:5,000 solution *in vitro* on *L. loa* is pronounced, as described by SHARP (1924)) There was also a reduction in microfilaria counts after injections in these cases, but controls showed so much variation in numbers circulating in the blood at different times that no conclusion could be deduced that the drug had any permanent beneficial influence *in vivo*

MOLSER (1939) had claimed that *A. perstans* disappears from the blood with intravenous injections of methylene blue, using 2 c c up to 10 c c of a 1 per cent solution This was contrary to our experience with either *A. perstans* or *L. loa* It was at this time believed, as HISSERTE (1938) stated, that it was highly improbable that any drug would be found that could be given in sufficient concentration to destroy filaria *in vivo* and spare the patient.

The situation has since been altered by new studies on the chemotherapy of these filarial diseases, and the discovery that the cotton rat (*Sigmodon hispidus*) can be infected in the laboratory with *Litosomoides carini* conveyed by the mite *Lyponissus bacoti*, so different drugs in higher concentration can now be experimented with on this filaria while it is living in its host This will be referred to later

A few patients infected with *L. loa* are now being given methylene blue by an intensive method, commencing with 5 c c of a 5 per cent solution

The effect of repeated injections of such dyes on the reticulo-endothelial system was described by CLAYTON LANE in 1937 The mobile cells of this system become blocked and their defensive mechanism thrown out of action by being overloaded with dye This suggests that, if methylene blue is to be successful, it must be given by intensive means in order to achieve a sufficient "knockdown" effect on the filaria, so that damage to the reticulo-endothelial system, and the resultant effect that the latter might have on the absorption of destroyed worms, can be avoided

In 1938 ADAMS found that the microfilaria of *O. volvulus* can be reduced in number by plasmoquine and neostibosan, with a temporary reduction of eosinophilia, although the adult was not affected Anthiomaline was the popular drug in the Belgian Congo before the war Only one case in a European in the Sudan was treated with this antimony preparation It was given on alternate days up to 3 c c of 6 per cent solution by injection, on the lines of DE CHOISY's method of 1937 Microfilariae were not seen in this case, but calabar swellings and the appearance of the worm in the eye were experienced from time to time Beyond making the patient feel rather ill, the drug had no effect

FILARIA NEGRO REIN TESTS.

Serial No.	Sex.	10 min.	6 hrs.	17 hrs.	48 hrs.	Nematode in blood.	Eosinophils.	Remarks.
1	ML	+	(+)	—	—	<i>A. persiana</i>	11	N intestinal or <i>Ancylostoma</i> . History of Calabar swelling. Some pruritus on 5th day <i>S. mansoni</i> No ov <i>S. mansoni</i> <i>Ancylostoma</i> No ov
	ML	—	—	—	—		20	
3	ML	+	+	+	(+)	and <i>L. loa</i>	40	
4	F	+	+	—	—	—	17	
6	F	+	+	+	+	<i>A. persiana</i>	42	
8	F	+	+	+	—		20	
		$\frac{1}{4}$ hr	2 hrs.	18 hrs.				
7	ML	+	(+)	(+)		<i>A. persiana</i>	28	<i>Ancylostoma</i> N ov <i>S. mansoni</i> <i>Ancylostoma</i> Not estimated <i>S. mansoni</i>
						and <i>L. loa</i>		
9	ML	—	—	—		<i>A. persiana</i>	19	
9	M	++	+	(+)			18	
10	F	++	+	—			16	
11	F	—	—	—			25	
12	ML	—	+	—			6	
13	ML	(+)	(+)	—		and <i>L. loa</i>	Not estimated	
14	F	+	+	—		<i>A. persiana</i>		
		$\frac{1}{4}$ hr	2 hrs.					
15	F	+	—			<i>A. persiana</i>	Not estimated	<i>Schistosoma</i> and <i>Ancylostoma</i> <i>Ancylostoma</i> N ov <i>Ancylostoma</i>
16	ML	+	(-)					
17	F	+	+					
18	M		(-)					
		15 min	$\frac{1}{4}$ hr					
19	F	—	()			<i>L. loa</i> and <i>A. persiana</i>	19	<i>Ancylostoma</i> No ov <i>Ancylostoma</i>
20	ML	()	()			<i>A. persiana</i>	18	
21	M		(+)			and <i>O</i>	20	
						<i>A. persiana</i>		
22	F	+					18	
23	F	()	(-)				23	
24	F		—				22	

Nos. 1-18 were done when the Antigen was fresh. 19-24 were done one week later.
(+) = weak positive.

Nos. 8 and 11 are negative, i.e. 8.3 per cent.

HUGH M. WOODMAN

reaction. All of his six *L. loa* cases gave positive results. Ninety-one per cent. of TALIAFERRO and HOFFMAN's (1930) *W. bancrofti* cases gave positive reactions. WRIGHT and MURDOCH (1944) found that 20 out of 20 cases of *O. volvulus* infection gave a positive reaction and, diluted to 1:4,000, seven out of a series of 19 were positive—a reaction which they found *Trichuris*, *Ascaris*, and other nematodes, also gave. These workers also found that cases of long exposure to *W. bancrofti*, and showing microfilaria in the blood, may give negative reactions, and these were assumed to be examples of desensitization.

Antigens have now been prepared and used for this test by CULBERTSON (1944) and OLIVER-GONZALEZ (1944) from microfilaria of *W. bancrofti*, from *L. carini* of the cotton rat (also by CULBERTSON), and from *Contortospiculum rhee* of the South American ostrich by MOHR and LIPPELT (1940). The interpretation of the reaction is confusing. Different workers seem to get different results, or put different interpretations upon their findings, the fundamental difficulty being that it is impossible to be certain that a resident subject in a filaria area has not been a victim of a nematode infection. If previously thus exposed, it is not yet clear to what degree intestinal nematodes influence the reaction given by filariids, or which species of the latter is the best for the group or for the more specific reaction.

When data have been collated from a bigger range of cases in the Southern Sudan which is now being undertaken, it is believed that its significance will be better understood, and it is expected to be of definite value in diagnosis of early filariasis in Europeans.

SUMMARY OF CONTENTS

The occurrence of *L. loa*, *W. bancrofti*, *O. volvulus* and *A. perstans*, and their distribution in the Sudan, is described.

Reference has again been made to the development of *L. loa* in *C. distinctipennis* and *C. longicornis* in the Southern Sudan. Evidence is put forward to suggest that there is another, and possibly commoner, vector of *L. loa* than *Chrysops*.

A survey of the incidence of *O. volvulus* on the southern fringes of the endemic blindness area, and where onchocercal eye conditions are rare, revealed a skin infection up to 77 per cent. of the population.

The therapeutic use of methylene blue, arsenic, antimony, antrypol, and other drugs, has been referred to.

Skin antigen tests with material prepared from avian filaria are described.

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THE INCIDENCE OF SCHISTOSOMIASIS IN SOUTH CENTRAL AFRICA *

BY

M GELFAND, M D, M R C P,

AND

W F. ROSS, B SC, M B, CH B,

From the Government Medical Service, Southern Rhodesia

A number of papers have been contributed from different parts of the world where schistosomiasis is endemic on the distribution and incidence of the disease. Its incidence or frequency in almost all instances has been determined by microscopical examination of the excreta. For instance, in Egypt, SCOTT (1937) conducted an extensive survey of the upper and lower Nile, and by examination of specimens of urine and stool described the geographical distribution and incidence of *Schistosoma haematobium* and *S. mansoni* infections. Briefly, he examined a single specimen of urine and stool from each patient, these specimens being collected at the same time and the collection being supervised to ensure as far as possible that they originated from the patients intended. Similarly, but on a smaller scale and with the same object in view, GOPSILL (1937), in Nyasaland, CAWSTON (1918), in South Africa, BLACKIE (1932), in Southern Rhodesia, BLACKLOCK (1930), in West Africa, carried out surveys by examination of urine and stools and published their findings.

This particular method is subject to several serious criticisms. In the first place, a single specimen, or even a few, is very often inadequate, and in such a survey the collection of more than one specimen, especially the stool, becomes for obvious reasons an almost impossible requirement. Secondly, in a survey of this nature one might easily collect one or other of the specimens required, but the second might not be forthcoming or be overlooked. Figures may therefore be inaccurate, so that when determining the incidence of *S. mansoni* or *S. haematobium* in a region it is desirable that both specimens from the same patient be examined. In the third place, the method adopted for the preparation of the specimen for microscopy also has some bearing on the final results, especially in the case of the stool examination.

* This is a further study undertaken to corroborate earlier and original work presented as an M D thesis by one of us (M G). Grateful thanks are due to Dr R M MORRIS, Secretary of Health, for permission to pursue this work, to Dr G R ROSS, Acting Secretary of Health, for permission to publish, and to Dr D M. BLAIR and staff of the Schistosomiasis Laboratory for help in the preparation of much of the material.

The method of the examination of the excreta does, however provide a rough idea as to the relative frequency of the two parasites and their geographical distribution. Its main advantage is that it is comparatively simple and large numbers can be dealt with over a short period of time. On the other hand, for the above reasons no accurate idea of the incidence in a country is possible unless a technique providing a higher figure of positive results is adopted.

Equally unsatisfactory would be rectal or bladder biopsies as the technique, especially in the latter is difficult and, when dealing with the primitive Africa peoples, admission to hospital is almost essential. In any event, whilst the biopsy technique provides a remarkably high percentage of positive results, there is a certain number of cases which are overlooked and once again errors in the figures must be expected. GELFAND (1947) found that snips taken of bilharzial bladders at autopsy did not give a sufficiently high proportion of positive results to justify the adoption of this relatively simple method of determining the incidence of *S. haematobium* infestation. His experience of rectal biopsies was similar.

For *S. mansoni* infestations KHALIL and SALAH EL DIN (1930) introduced a method of scraping the rectum using the gloved finger but whilst this procedure may give a higher percentage of successes than simple macroscopical examination of the stool, yet it fails to reveal all the cases. WELLEN (1947) introduced, as a modification of this, his rectal scraper for the diagnosis of *S. mansoni* and derived fairly accurate results. However in Southern Rhodesia, MEYER, ROSE and BLAIR (1948) found this technique to be quite unsatisfactory. These procedures are of interest and of use in the diagnosis of the individual case, but are unsatisfactory for large-scale investigations.

It should be clear that the digestion of the entire rectum and bladder in each case at autopsy would provide the most accurate idea as to the presence or absence of the disease, since these two sites are practically always affected. It is true that a certain percentage of cases would be overlooked where the ova are deposited in areas other than the bladder or rectum, and therefore this method would not give an exact incidence. However these aberrant or ectopic deposits are rare as in the last 100 cases, in which only the bladder and rectum were examined, 89 cases showed the disease, and one can therefore assume this event to be uncommon. On the other hand, in the first 50 cases in which other viscera were studied, two showed ectopic deposits. Similarly these figures show how uncommon unisexual infestation must be as ova are so frequently present.

The method of digestion of the entire organs at autopsy has many advantages, for should ova be present, they will almost certainly be found by this method, while by other techniques, as already shown, they cannot be demonstrated in not infrequent number of schistosomal subjects. It also provides an accurate idea of the frequency of both infestations and overcomes the difficulty of collecting stools. However this method being applicable only to

autopsies, gives the incidence in these cases which have been in hospital or autopsied for medico-legal purposes. Also, as the work is being carried out in a large European centre, the majority of patients examined are adult males, the women remaining on the whole in their reserves.

A disadvantage of this method is the time required for the collection of a sufficiently large series of cases. If the disease was infrequent, then a very large number of cases, probably several hundred, would be required. However, when one finds that practically every autopsy shows evidence of the disease, no matter from where the deceased comes, whether it be from a village in Nyasaland, Southern Rhodesia, Northern Rhodesia, Portuguese East Africa or Portuguese West Africa, then the inference is that the disease must be extremely prevalent. If, for instance, in 100 cases only 20 had the infection, then it would be necessary to determine if these infected cases were located in any one particular area and the rest of the territory was free from infection. The second disadvantage is that whilst ova are found one cannot say whether at the time of death the disease was in an active state or whether it had been an old infection, nevertheless, the fact that there were ova shows that the patient was affected by the pathological effects of a schistosome infection to a varying degree of severity, being slight in some but in others marked. A further disadvantage is that this method requires laboratory facilities and trained staff to carry out the extensive preparation of specimens and the microscopical work required to examine these.

It will be seen later that this series of cases covers a vast area of Central Africa from the Limpopo, across the Zambesi to North Eastern Rhodesia, including Lake Nyasa, and from the Indian Ocean west almost to Angola. The majority of the cases originate from what might be described as British South Central Africa.

METHOD

In this series adult Africans, both male and female, from the ages of 18 to 60 were examined. The majority were between 20 and 35 years of age. Most of the cases were males but, nevertheless, the female incidence of infection appeared as high as that of the males.

Autopsies were unselected, and no matter what the cause of death, whether by accident or disease, the cases were examined routinely and consecutively for schistosomiasis.

The urinary tract, including the kidneys, ureters and bladder, was carefully dissected out, after which the rectum up to the sigmoid colon was removed. The bladder was opened by a vertical incision on the anterior surface through the urethral passage and inspected for gross pathological changes. The ureters were next divided at their entry to the bladder, and placed in a glass jar. A similar procedure was carried out with the rectum. The glass jars were then labelled with the name of the deceased and the organ contained therein. No fixative was added, and the specimens were taken immediately to the Schistosomiasis Laboratory.

On arrival, the bladder and rectum were each cut up into small pieces and were then placed in separate glass jars, covered with 10 per cent potassium hydroxide and placed

in an incubator at 37 C. for 24 hours. The jars were then removed and the contents were poured through a tea strainer to keep back the gross material not yet "digested." The filtrate was then transferred to centrifuge tubes and spun in an electric centrifuge at 1 000 revolutions per minute for 2½ minutes. The supernatant fluid was decanted, and the deposit tapped out on to a slide covered with a cover-slip and then examined microscopically. Four such slide preparations were made and each studied carefully with the 2/3-inch lens for ova. If no ova were found in four slides the specimen was considered negative. This procedure was carried out for each organ.

RESULTS.

We examined at autopsy 150 adult Africans. One hundred and fifteen of these were males and 33 were females.

The country from which they originated was determined and the distribution was 53 from Southern Rhodesia, eight from Northern Rhodesia, 53 from Nyasaland, 21 from Portuguese East Africa, seven from Portuguese West Africa and in eight we were unable to obtain information as to the country of origin.

Of the 150 cases examined, 147 (98 per cent.) were found to suffer from schistosomiasis, using the previously described method of examination, 113 males and 34 females being positive. The number of positive cases from each country is shown in the table.

TABLE
SHOWING COUNTRY OF ORIGIN AND NUMBER OF UTERI FOUND POSITIVE.

	S.R.	N.R.	Nyas.	P.E.A.	P.W.A.	Unknown.	Total.
No. from country	53	8	53	21	7	8	130
No. positive	52	7	53	21	7	8	147
Percentage positive	98.1	87.5	99.1	100	100	100	99

It was found that out of the 147 positive cases, 146 had *S. haematobium* infection (99.3 per cent.) 72 had *S. mansoni* infection (48.9 per cent.) and 71 (48.3 per cent.) had both *S. haematobium* and *S. mansoni* infection.

The distribution of *S. haematobium* in the bladder and rectum was as follows. *S. haematobium* was found in the bladder in 134 autopsies, in the rectum in 112 autopsies, in the bladder but not in the rectum in 22 autopsies, and in the rectum but not in the bladder in eight autopsies. Similarly *S. mansoni* was found in the rectum in 67 autopsies, in the rectum and bladder in five autopsies, in the rectum but not in the bladder in 62 autopsies, and in the bladder but not in the rectum in three autopsies.

We also recorded a comparison of the results following "digesting" the whole viscus and the examination of a small portion of the mucous membrane lining the viscus prior to its "digestion." We found in the series of 150 cases

that whereas 134 bladders were positive on digestion, 117 gave positive snips. Similarly, out of 137 rectums from patients suffering from schistosomiasis, 107 rectal snips were positive.

DISCUSSION

This series of 150 consecutive autopsies of Africans from a wide area of South Central Africa shows how widespread is schistosomiasis. All other records to date dealing with the incidence of the disease in this area of Africa failed to show its very high degree of prevalence. BLACKIE (1932), in his Helminthological Survey of Southern Rhodesia, found by examination of stools and urine a varying incidence of 10 to 30 per cent for indigenous natives, and from 35 to 63 per cent for alien natives. Similarly, GOPSILL (1937), in Nyasaland, did not demonstrate such a high incidence as was found in this investigation (70 per cent for *S. mansoni* and 30 per cent for *S. haematobium*).

If this work is confirmed by others it would tend to confirm our belief that there would appear to be little use in searching for ova in the stool and urine specimens taken from patients admitted to hospital in South Central Africa, as one might justifiably assume that the disease is present or has been present in almost every case. This is not intended to mean that a careful examination of the stool and urine microscopically should not be undertaken. If this were not done in the case of the stool, hookworm and other helminthic infections would be overlooked and if no urinary examination took place the presence of urinary casts, blood and other deposits which are of great clinical significance, would be missed. The point we wish to stress particularly is that a negative result should not be accepted as indicating the absence of the disease, and one might justifiably assume that practically every African from this part of Africa has or has had the disease.

When ova are deposited in a viscus some tissue reaction occurs proportional to the number of ova deposited, and occasionally this may lead to serious consequences even though the infection be no longer active. For example, it is well known that once ova are deposited in the ureteric wall the inflammatory changes and subsequent fibrosis may continue long after the infection is dead and the development of stricture or dilatation may ensue.

The results show that *S. haematobium* is by far the commoner of the two parasites, and this has been confirmed by other workers in this part of Africa.

The autopsy figures stress how much more common the two infections are than had previously been considered. They also reveal how infrequently a pure *S. mansoni* infection occurs without the presence of *S. haematobium* at the same time, in fact, only one case of pure *S. mansoni* infection was found. It would appear that in South Central Africa, *S. mansoni* is almost always accompanied by *S. haematobium*.

A useful result of this work is that the snips removed from rectum and bladder at autopsy confirm our findings in the living that as a method of determining the incidence of the disease, it is not very satisfactory, although it does

provide a high figure of accuracy. For example, the bladder snips were positive in 66 per cent. as compared with digestion of the whole organ, and the rectal snips were positive in 78 per cent. as compared with digestion of the rectum. It also shows that it is possible to diagnose *S. haematobium* infestations by rectal biopsy. In this particular series 92 rectal snips (68.6 per cent.) were positive for *S. haematobium*, in 134 cases showing positive *S. haematobium* infections in the bladder. Thus *S. haematobium* can be fairly readily diagnosed by this technique which will be further discussed in a subsequent paper.

SUMMARY

1. The incidence of schistosomiasis was determined at autopsy in Salisbury Southern Rhodesia.

2. The bladders and rectums from 150 adult Africans were removed *in toto* and digested in potassium hydroxide, small snips of mucosa having previously been removed and examined microscopically for ova.

3. Of 150 subjects from widely distributed areas of South Central Africa the digested deposit showed 93 per cent. positive for ova of *S. haematobium* and *S. mansoni*. Of these, 147 had *S. haematobium*, 72 had *S. mansoni* and 71 had both *S. haematobium* and *S. mansoni*.

4. Only one case of pure *S. mansoni* infestation was found which does not bear out clinical experience.

5. Evidence is submitted that there must be few unisexual infestations.

6. The high incidence of the disease in South Central Africa is stressed and is far greater than simple microscopical examination of stool and urine would imply.

7. A comparison of rectal and bladder snips with digestion results is also given. In 134 cases showing positive bladder snips for *S. haematobium* ova, 92 (68.6 per cent.) rectal snips were positive for *S. haematobium* ova.

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OBSERVATIONS ON THE TREATMENT OF FALCIPARUM MALARIA

BY

ROBERT H. BLACK,*

Department of Tropical Medicine Liverpool School of Tropical Medicine

The introduction of modern antimalarial drugs such as paludrine and chloroquine has tended to divert attention from quinine and its activity against *Plasmodium*. Indeed, so much is this the case, that the intravenous injection of paludrine has been used even for the treatment of cerebral malaria! The purpose of this communication is to outline a rational view of the specific treatment of falciparum infections in the light of our present knowledge of the mode of action of antimalarial drugs.

In a previous communication (BLACK, 1946) it was reported that antimalarial drugs, in the form in which they circulate in the blood, produce definite lethal effects on trophozoites of *Plasmodium falciparum* developing in cultures. Previous workers had found that antimalarial drugs added to cultures of *Plasmodium* caused little or no effect on the subsequent development of the parasites. It has been shown, however (BLACK, 1946), in the cultures of *P. falciparum* with media containing paludrine in the form in which it circulates in the body, the parasites continue to develop in parallel with those in control cultures containing no paludrine, until they reach the early schizont stage. Then development stops and the parasites become vacuolated and degenerate. In cerebral malaria the parasites anchored within the cerebral capillaries are in the later stages of development. Since paludrine exerts its action upon trophozoites only at the stage of earliest chromatin division, much time will necessarily be lost in the treatment of a case of a heavy falciparum infection if paludrine is the only drug used.

* Working under a grant from the Medical Research Council

Quinine and atebuin, in cultures of *P. falciparum*, arrest the development of the parasites early in the asexual cycle. The following table shows the results seen in cultures containing quinine, atebuin and paludrine, and contrasts the site of attack of the different drugs on the various stages of development of the parasite. Quinine, acting on the ring and amoeboid stages of development of *Plasmodium*, will prevent the parasites from developing to the early schizont stage—which paludrine will not do. The pathological changes in the brain in cerebral malaria are associated with the presence of parasitized red cells—at the pre-schizont stage of development, and later

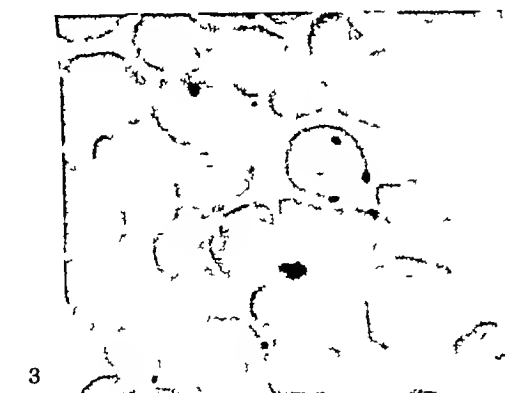
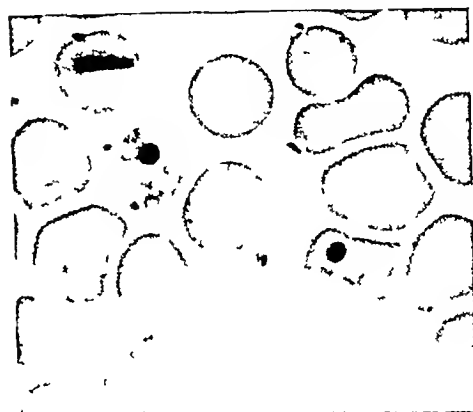
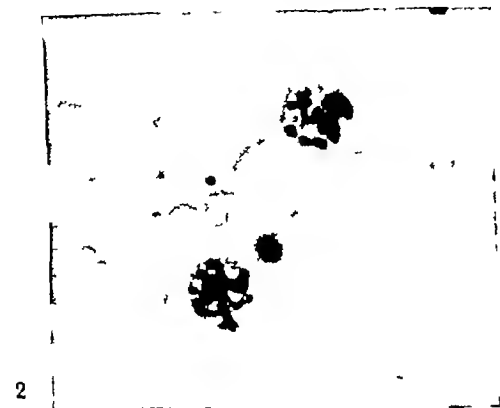
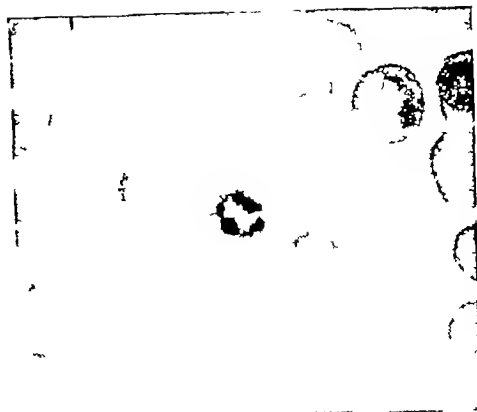
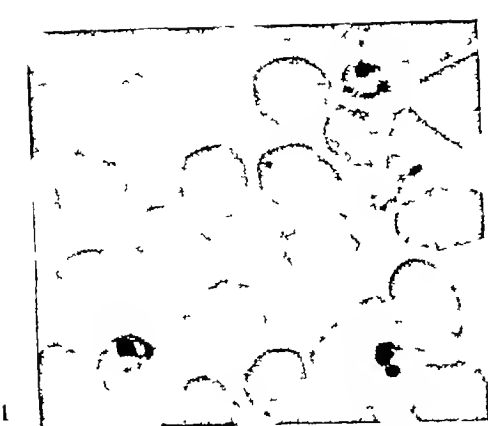
Drug	Serum concentration or dosage régime.	Strain of parasite	Forms seen in culture samples.						
			R	A	Ps	Es	Ds	Ms	R ₂
Quinine	4 mg./litre	11AR	+	—	—	—	—	—	—
Atebui	300 γ/litre	11AR	+	—	—	—	—	—	—
Paludrine	1 bc. after glucose 0.1 by mouth	11AR	+	+	+	—	—	—	—

Ateb — R rings, A amoeboid forms, Ps pre-schizonts, Es early schizonts, Ds divided schizonts, Ms mature schizonts, R₂ rings of second generation, + forms in culture — (forms not developing. (New Guinea strains of *P. falciparum*.)

—blocking the smaller vessels. Since paludrine does not prevent the parasites from developing to the stage where they will lodge in the brain capillaries, quinine, which acts on the early ring and amoeboid forms of the parasite, should be used in the treatment of cerebral malaria as well as in heavy infections with *P. falciparum*. Indeed, STATION (1948) reports the case of a patient with a heavy *falciparum* infection who went into a coma some time after the administration of paludrine mg. 400 given over a period of 2 days and recent unpublished work shows that death may occur in some heavy *falciparum* infections despite the intravenous injection of paludrine.

Another feature seen during the treatment of malaria with paludrine is the occurrence of an after-temperature. When paludrine is given for the treatment of *falciparum* infections the 48-hours periodicity of the rise in temperature often continues for one further cycle. The rigor in human malarial infections has not yet been satisfactorily explained, but it is known to occur in association with schizogony.

The rapid control of the fever by quinine in *falciparum* infections may be contrasted with this after-temperature seen with paludrine. This after-temperature is probably related to the effect of paludrine upon the parasites, which takes place in the later stage of the schizogony cycle—the parasites, halted in their development just before schizogony degenerate and a rise in



FIGS 1-3 Development of *P. falciparum* in normal control cultures containing no drug in the serum of the medium

FIGS 4-6 Samples from a culture containing paludrine in the serum of the medium

The two cultures were commenced at the same time. The three pairs of samples were taken after 21.5, 38.5 and 41.5 hours' incubation

Aitape Wewak (New Guinea) strain of *P. falciparum*, found it more resistant to atabrin suppression than strains from other areas of the same island. BLACKIE (1947) stated that some of the African strains of *P. falciparum* did not respond to treatment with paludrine in the manner described for other strains of the same parasite and variations in the effect of antimalarial drugs upon plasmodia in bird malaria was demonstrated by LOURIE (1934), who showed that *P. relictum* from Germany showed a considerable difference in its response to quinine from that of a North American strain. Thus strains of the species of parasites of human and bird malaria differ amongst themselves in their response to antimalarial drugs.

The value of quinine in the treatment of severe falciparum infections should be re-emphasized. The best treatment for a good therapeutic response is the intravenous injection of a solution of quinine dihydrochloride grain 10 given over a period of 10 minutes. In debilitated patients with thiamin deficiency however the injection may have to be given more slowly—as by an intravenous drip infusion (STRAHAN 1948). But it is felt that for the usual cases of heavy falciparum infection and cerebral malaria the intravenous injection of quinine followed by oral paludrine, is the correct treatment. The value of quinine is demonstrated by an experience at the Cairns (Australia) Medical Research Unit of which the staff volunteered for some final experiments with paludrine and falciparum malaria after the recent war had ended (FAIRLEY *et al.*, 1948b). One of the professional staff developed malaria and was treated with oral quinine for 2 days followed by paludrine. The response to treatment was good.

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A NOTE ON PRESUMED EXO-ERYTHROCYTIC
DEVELOPMENT OF *PLASMODIUM VASSALI* IN THE
LIVER OF THE MALAYAN SQUIRREL *

BY

JOHN W FIELD,

AND

J I B EDESON

From the Institute for Medical Research, Federation of Malaya

A pigmented parasite in the red blood cells of the squirrel was first described by VASSAL (1905) in Indo-China. LAVERAN (1905) gave it the name *Haemamoeba vassali*. A similar parasite was seen by DONOVAN (1920) in India and by GREEN (1933) in Malaya, and studied in the Malabar squirrel by MULLIGAN and SOMERVILLE (1947). The parasite somewhat resembled *Plasmodium malariae* in man, but differed sharply in that dividing forms were never seen in the red blood cells. The site of schizogonic multiplication was not known.

* Dr P C C GARNHAM has examined some of our material and sent us for comparison a specimen of *Plasmodium kochi* (*Hepatocystes kochi*) in the monkey liver. We thank him for his interest and help. We are indebted to Dr SAVOOR for the preparation of tissue sections, to the Scrub Typhus Research Team now working in the Institute for the supply and identification of the squirrels, and to Mr YAP LOY FONG for the drawings reproduced in the plate.

The known pattern of exo-erythrocytic development in the malaria parasites of mammals is still fragmentary and further study of this squirrel parasite might, it was thought, be useful. Serial examinations quickly established that infection is common in at least five species of Malayan squirrel that some of the infections are extremely heavy and that wherever schizogony occurred it was not in the red cells of the peripheral blood. The blood forms resembled *Haemamoeba vanah* now known as *Plasmodium vanah* closely enough to suggest that the parasite was probably the same.

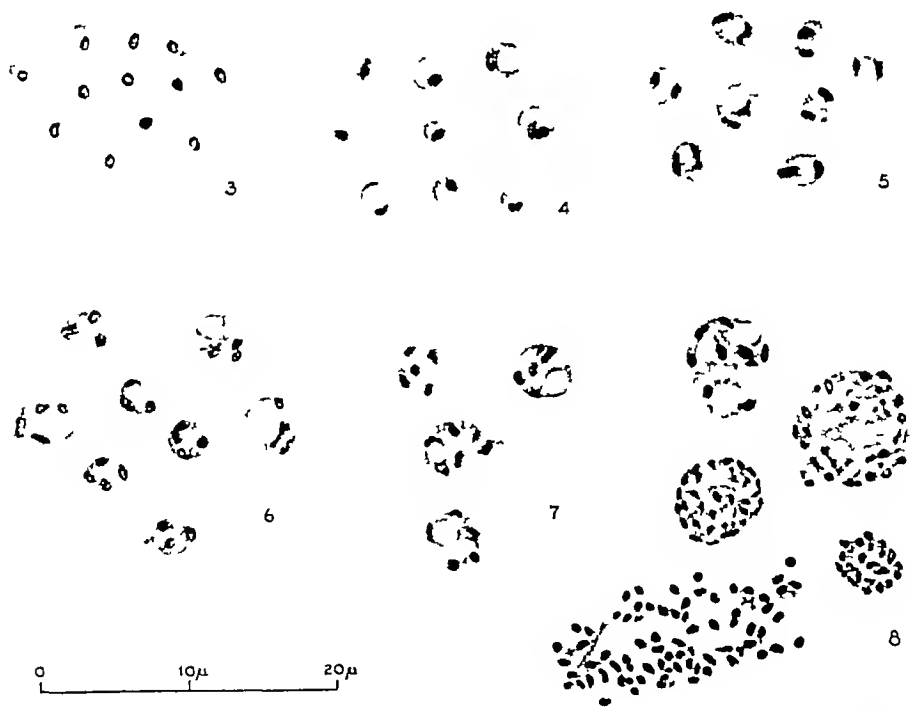
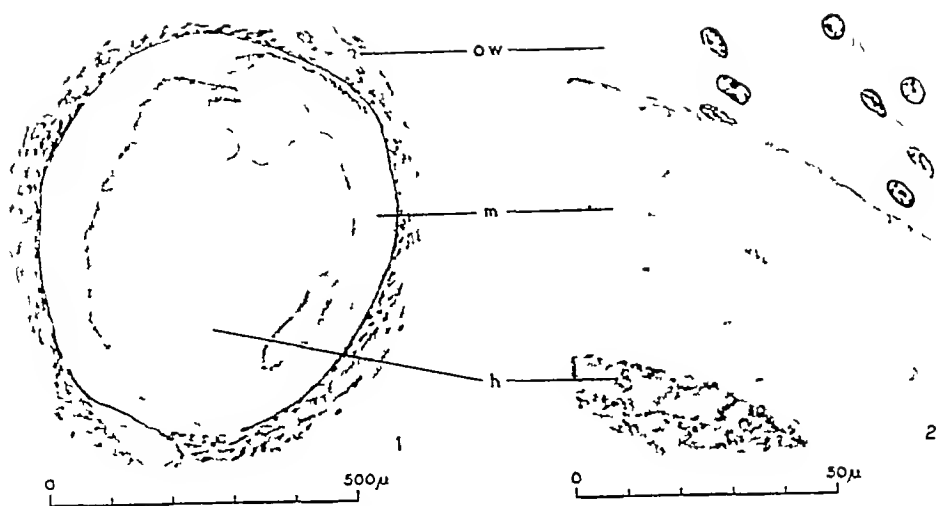
TABLE
INCIDENCE OF INFECTION WITH MALARIA-LIKE PARASITE IN
VARIOUS SPECIES OF MALAYAN SQUIRREL.

Species of squirrel.	Number examined.	Number infected.
<i>Callosciurus notatus</i>	47	20
<i>myristicatus</i>	27	27
<i>lutescens</i>	17	18
<i>caniceps</i>	4	4
<i>Lariscus banyani</i>	4	
Total	79	69

DESCRIPTION OF PLATE:

The drawings were made at the microscope from Giemsa-stained smears and sections of liver from animals infected with *P. vanah*.

- 1 Section of cyst from the liver of young squirrel with a heavy blood infection. There were six cysts in the section. ($\times 85$ approx.)
 - a — Outer wall of cyst composed of compressed liver tissue. This zone sometimes contains giant cells and groups of inflammatory cells not shown in this particular cyst.
 - m — Zone of tightly-packed merozoites normally in contact with liver substance but here partly detached from shrinkage during fixation.
 - b — Amorphous material filling the interior of the cyst.
- 2 Portion of cyst at higher magnification. ($\times 700$ approx.).
- 3-8 Merozoites and extra-cellular dividing forms from Giemsa-stained smears prepared by crushing isolated cysts between two slides. ($\times 2,000$ approx.).
- 3-4 Isolated merozoites with little visible cytoplasm (3) or with cytoplasm clearly seen (4).
- 5-7 Extra-cellular dividing forms with from 2 to 8 nuclei.
- 8 Large extra-cellular schizont-like collections of merozoites.



Yap Joo Fong

Presumed exo-erythrocytic stages in the development of *P. vassali* in the squirrel liver

The known pattern of exo-erythrocytic development in the malarial parasites of mammals is still fragmentary and further study of this squirrel parasite might be useful. Serial examinations quickly established that infection is common in at least five species of Malayan squirrel that some of the infections are extremely heavy and that wherever asexual development occurred it was not in the red cells of the peripheral blood. The blood forms resembled *Haemamoeba venalis* now known as *Plasmodium venalis* closely enough to suggest that the parasite was probably the same.

TABLE.
INCIDENCE OF INFECTION WITH MALARIA-LIKE PARASITE IN
ABOUT SPECIES OF MALAY SQUIRREL.

Species of squirrel.	Number examined.	Number infected.
<i>Callosciurus notatus</i>	37	30
<i>apricinus</i>	37	37
<i>lunatus</i>	17	18
<i>sinensis</i>	4	4
<i>Lariscus major</i>	4	
Total	110	89

DESCRIPTION OF PLATE.

The drawings were made at the microscope from Giemsa-stained smears and sections of liver from animals infected with *P. farshi*.

- 1 Section of cyst from the liver of young squirrel with heavy blood infection. There were six cysts in the section ($\times 85$ approx.).

a.w — Outer wall of cyst composed of compressed liver tissue. This zone sometimes contains giant cells and groups of inflammatory cells not shown in this particular cyst.

m. — Zone of tightly-packed merozoites normally in contact with liver substance but here partly detached from shrinkage during fixation.

b. — Amorphous material filling the interior of the cyst.

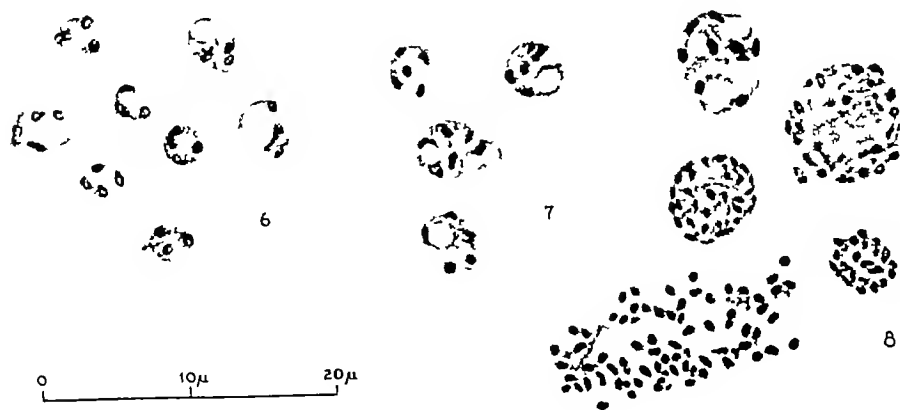
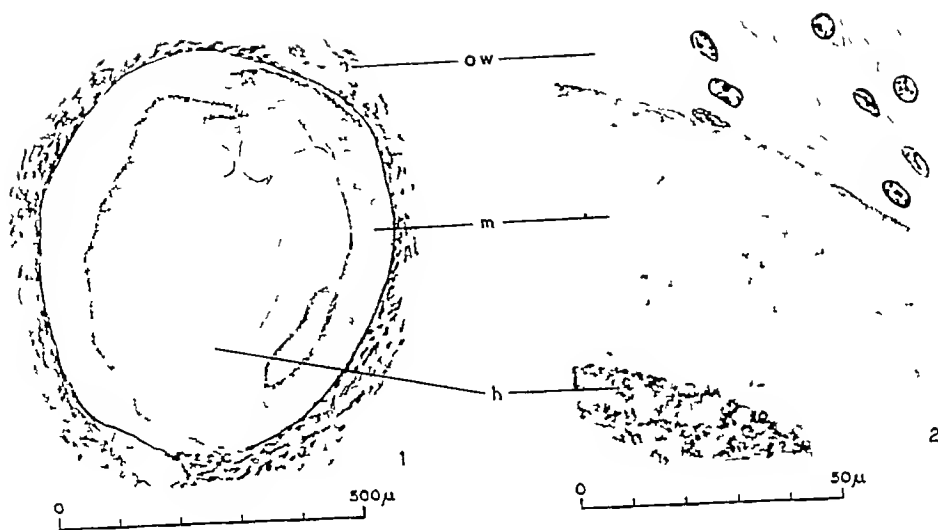
- 2 Portion of cyst at higher magnification. ($\times 700$ approx.)

3-8. Merozoites and extra-cellular dividing forms from Giemsa-stained smears prepared by crushing isolated cysts between two slides. ($\times 1000$ approx.).

3-4 Isolated merozoites with little visible cytoplasm (3) or with cytoplasm clearly seen (4).

5-7 Extra-cellular dividing forms with from 3 to 8 nuclei.

8 Large extra-cellular schizont-like collections of merozoites.



Chap. Ioy fong

Presumed exo-erythrocytic stages in the development of *P. vassali* in the squirrel liver

Schizogony in the red cells was not seen in some hundreds of films examined, nor was there unequivocal evidence of schizogony in smears from brain, lung, spleen, bone-marrow and kidney. Smears made haphazardly from the liver were also negative, but an examination of the liver surface, prompted by GARNHAM's discovery that schizogony in *P. kochi* in the lower African monkeys occurs in liver cysts which are visible to the naked eye, revealed the presence of cysts on the liver surface in many of the infected animals. Smears from these cysts showed innumerable merozoites.

The cysts varied in size to a maximum observed of 840μ ; many were thus visible to the naked eye. They were smooth, round and translucent and could be detached fairly easily from the liver substance with a pair of sharp needles, under a dissecting microscope. They were seen most easily on the liver surface but occurred also throughout the organ. Giemsa-stained smears contained an immense number of merozoite-like structures, mostly single but also in rounded, extra-cellular collections of a dozen to fifty or more. There were also extra-cellular dividing forms with two to six or more nuclear segments. In sections the cysts had an outer wall of compressed liver parenchyma. There was little tissue reaction as a rule but the outer wall sometimes contained giant cells or groups of inflammatory cells. Within this outer wall of compressed liver tissue there was a zone up to 50μ or more wide packed tight with what appeared to be merozoites. This zone was limited externally by a thin membrane which was sometimes in contact with the outer wall but more often detached from shrinkage of the cyst during fixation. The inside of the cyst contained amorphous material with vague condensations suggesting degenerate merozoites.

We have compared these cysts with the merocysts of *P. kochi* in a section of monkey liver sent to us by Dr GARNHAM. The resemblance is very close, and it seems that the mode of exo-erythrocytic development of *P. vassali* in the squirrel liver and of *P. kochi* in the liver of the monkey may well be the same.

The systematic position of this parasite is uncertain. Do the schizonts in the liver and the trophozoites and gametocytes in the blood represent stages in the life history of the same parasite? The blood forms resemble those of *P. vassali*, the cysts in the liver are very like those of *P. kochi*—a parasite now placed by GARNHAM (1948) in a new third genus of the family Haemoproteidae, the genus *Hepatocystes*, so called because of the distinctive cyst-like liver schizonts. Should *Plasmodium vassali* more correctly be named *Hepatocystes vassali*? What is the relation between *P. vassali* and the blood parasite recently described by RAY (1948) in the Himalayan flying squirrel (*Petaurista* sp.)—a parasite which also produces cyst-like schizonts in the liver? These and other questions await further study.

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RATE OF DISAPPEARANCE OF *LEISHMANIA* IN KALA- AZAR PATIENTS UNDER UREA STIBAMINE THERAPY.

BY
EUTROPE A HO
TSUNG-HSIN SOONG

AND
YOUNG LI,
National Institute of Health, M O H, Nanking, China

Although pentavalent antimony has been used for treating kala-azar for more than 20 years, adequate knowledge concerning the rate of disappearance of *Leishmania* from patients under treatment is lacking. Likewise the proper adjustment between the dosage of drug and the degree of infection has been insufficiently studied. The common practice, in the case of urea stibamine, as well as other antimony drugs, is to administer the drug to a patient continuously, usually every other day, in 10 to 20 injections, until a certain arbitrary total amount is reached and a clinical improvement obtained. Apart from being empirical, this practice often causes toxic complications from over-dosage.

As far as the authors are aware, attempts to determine the degree of infection in patients prior to treatment have never been reported in the literature. Conflicting reports concerning the potency of a given drug were often encountered. The *Chinese Medical Journal* noted (1931) that there was a great variation in the amounts of neostibosan used by different hospitals for treating kala-azar patients, which varied from gramme 2 to 6 per case. In the case of urea stibamine, Ho (1944) reported a cure could be obtained with a small amount given in six weekly injections.

In this study experiments were carried out to determine the relationship between the degree of infection prior to treatment and the rate of disappearance of *Leishmania* on one hand, and the frequency of injections with which the drug is administered on the other. It was hoped results could be obtained that might explain the dosage variation reported in the literature, and might be useful in establishing antimony therapy on a more rational basis.

METHOD

The individual dosage of urea stibamine employed in this study was kept constant, but the injections were given either once a week or twice a week. The dosage was based upon that recommended by Ho (1944), with slight modification. The total amount per case was gramme 0.04 per kg of body

weight for infants, grammes 0.03 per kg. for children, and grammes 0.03 per kg. for adults, to be evenly divided into six weekly or twice-weekly doses for intra venous injection.

The patients were divided into two groups—one group received injections once a week, and the other group twice a week. The grouping was made in the majority of cases, according to odd and even registration numbers. However during the latter half period of the study it was our practice to give patients with poor general condition weekly injections in order to avoid toxic reaction. All patients were treated as ambulatory cases. Except sulpha drugs, which were used in a few cases complicated with pneumonia and diarrhoea, no other supplementary treatment was prescribed, even in cases complicated by severe anaemia or noma.

The diagnosis in each case was established by finding *Leishmania* in smears prepared from material obtained by sternal puncture. The number of the parasites, if present, was counted and indicated arbitrarily by the following plus markings, thus showing to a certain extent the degree of infection prior to treatment

- (+) Indicated less than one parasite in 10 microscopic fields, magnification 450
- (++) Indicates 1 to 8 parasites
- (+++) Indicates 10 to 50 parasites
- (++++) Indicates more than 50 parasites

During the course of treatment, each patient was periodically re-examined for persistence or disappearance of *Leishmania* by means of sternal punctures which were done just before the third and the fifth injection, and 1 week or less after the sixth injection. A negative result was confirmed by a subsequent puncture.

The reasons why sternal puncture was used, instead of spleen or liver puncture, are as follows: first, although *Leishmania* are more readily found in spleen puncture, the number of the parasites present in the bone marrow smear prior to treatment, is more or less in proportion with the degree of infection; second, the parasites in bone marrow seem to be more resistant to treatment. See reference (3) for detailed information. Post treatment follow up for relapses was made.

INVESTIGATION AND RESULTS

(i) Rate of Disappearance of *LEISHMANIA*.

During a period of 2 years, from July 1944 to June, 1946 793 proved cases of kala azar in North west China were treated with ursa subamine in the dosage mentioned above. Of this total 499 patients received treatment once a week and 344 twice a week. The degree of infection prior to treatment in patients of each group was determined by the number of *Leishmania* present in bone marrow smears and indicated by plus markings. The result of the treatment is shown in Table I and Graph. It will be noted that the rate of

EUTROPE A HO TSUNG-HSIN SOONG AND YOUNG LI

disappearance of parasites from a patient under treatment (with relation to the number of injections) depends upon two factors (1) the number of *Leishmania* present in bone marrow smear prior to treatment, (2) the frequency of injections with which the drug is administered

TABLE I

THE RATE OF DISAPPEARANCE OF *Leishmania* FROM THE BONE MARROW IN RELATION TO THE NUMBER AND FREQUENCY OF INJECTIONS OF UREA STIBAMINE, IN 793 KALA-AZAR CASES WITH VARIOUS DEGREES OF INFECTION

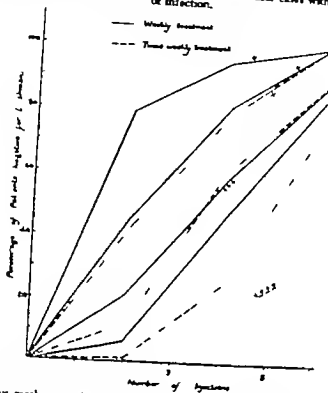
Result of examination for <i>Leishmania</i>	Weekly			Twice-weekly		
	After specified number of injection			After specified number of injection		
	2nd	4th	6th	2nd	4th	6th
Group 1*						
Number of patients examined	18	19	10	5	5	5
" negative	18	19	10	5	5	5
Per cent	100 0	100 0	100 0	100 0	100 0	100 0
Group 2*						
Number of patients examined	84	148	167	60	112	149
" negative	66	140	166	29	87	117
Per cent	78 0	94 6	99 4	42 1	77 7	98 3
Group 3*						
Number of patients examined	76	110	121	51	88	103
" negative	34	88	119	0 -	52	90
Per cent	44 7	80 0	98 4	11 8	60 2	87 4
Group 4*						
Number of patients examined	55	93	107	58	74	81
" negative	12	54	94	1	10	01
Per cent	21 8	58 0	87 8	1 7	25 7	75 3
Group 5*						
Number of patients examined	15	31	35	31	34	36
" negative	1	14	29	0	5	12
Per cent	6 7	54 2	82 0	0	14 7	33 3

* Groups 2 3 4 and 5 consisted of patients with (-) (+) (+-) and (+++) *Leishmania* in bone marrow smears, prior to treatment, respectively. Group 1 consisted of patients in whom parasites were not found in the bone marrow, but in the spleen prior to treatment. The definitions of these plus markings are given in the text.

Concerning the first point, in cases in which *Leishmania* were not found in the bone marrow prior to treatment, the parasites in the spleen would disappear after one or two injections, as observed in 24 such cases. However, when the parasites were present in the bone marrow, as in the remaining 769

GRAPH.

The rate of disappearance of *Leishmania* from the bone marrow in relation to the number and frequency of injections of urea stibamine in 793 kala-azar cases with various degrees of infection.



Note—Plus markings indicate the number of parasites in the bone marrow prior to treatment as defined in the text.

cases especially when they occurred in large numbers, more injections were required to make them disappear. With (+) *Leishmania* in bone marrow prior to treatment, weekly treatment made the parasites disappear in 78.6, 84.6 and 99.4 per cent. of patients after the second, the fourth and sixth injection respectively. With (++) *Leishmania*, the corresponding figures were reduced to 44.7, 80.0 and 98.4 per cent. The figures were further reduced to 21.8, 58.0 and 87.9 per cent. for patients with (+++) *Leishmania* and even more so for patients with (++++) *Leishmania*. In the group of patients who received twice-weekly injections a generally similar trend was observed, although twice weekly injections were much less effective. These differences in the rate of disappearance of parasites were statistically significant.

Regarding the second point, it is easily seen in the Graph that for a given total dosage of urea stibamine to patients with comparable numbers of parasites

in the bone marrow prior to treatment, weekly injections gave a much better result than twice-weekly injections. When the parasites were present only in the spleen or present in very few numbers in the bone marrow, weekly or twice-weekly treatment did not show a difference in results, because in such cases the parasites would disappear after one or two injections. However, when the parasites were present in moderate or large numbers in the bone marrow, weekly injections undoubtedly gave better results than twice-weekly injections. The difference between the percentage of cases in these two groups in which *Leishmania* disappeared after a specified number of injections, was found to be statistically significant.

(11) *Correlation of Disappearance of LEISHMANIA and Blood Picture*

Anaemia, which was observed in practically all cases, improved steadily during the course of treatment. Table II shows the erythrocytic counts of those patients in whom complete counts at the end of one course of treatment could be made. It shows that an increase in the frequency of injections will not hasten the improvement of the red blood picture and that proper spacing of injections is desirable.

TABLE II
COMPARISON OF ERYTHROCYTIC COUNTS IN WEEKLY AND TWICE-WEEKLY TREATMENTS
AT THE END OF ONE COURSE OF UREA STIBAMINE

Groups	Number of cases	Counts in millions	
		4.5 or more	Less than 4.5
Weekly	191	51 per cent	49 per cent
Twice weekly	95	30	70

I eucopenia was observed in about 90 per cent of our cases. It improved steadily as the parasites gradually disappeared from patients under treatment. Table III shows the main leucocyte counts, prior to, during, and after treatment. Prior to treatment, the main counts for the weekly and the twice-weekly groups were $5,072 \pm 122$ and $5,289 \pm 140$ respectively, showing that they were comparable. At the end of one course of treatment, the figure for the former group increased to $9,299 \pm 162$ and that for the latter group to $7,557 \pm 153$. The difference between these two figures is 7.7 times their standard error, and is considered to be statistically significant. This clearly shows that an increase in the frequency of injections cannot hasten the disappearance of leucopenia. Unfortunately, there were not enough data available to find out whether the leucocyte count of the twice-weekly group would reach the level of that of the weekly group 3 more weeks after treatment. However, when the counts

in the weekly group were made just before the fourth injection and compared with those of the twice-weekly group determined at the end of one course of treatment, the difference between the two was negligible. The main counts, as shown in Table III were $7,844 \pm 213$ and $7,557 \pm 153$ respectively.

TABLE III.

LEUCOCYTE COUNTS OF SALAZAR PATIENTS WHO RECEIVED 5 FM OR TWICE-WEEKLY INJECTIONS OF UREA STIBAMINE.

Groups.	Number of cases.	Count					
		Before treatment.		During treatment.		After treatment.	
		Mean.	S.D.	Mean.	S.D.	Mean.	S.D.
Weekly	49	8,073 \pm 122	8.1	7,941 \pm 13		8,279 \pm 182	2.351
	23	8,613 \pm 16	2.319				
Twice weekly	350	7,299 \pm 160	2.811			7,557 \pm 153	2.461

(iii) *Correlation of Disappearance of Leishmania with Relapses.*

Table IV shows the number of relapses among patients who were free of the parasites at cessation of treatment. The period of follow-up extended to June 1947 1 year after the last case was treated. During a period of 3 years, 4.1 per cent. of patients in the twice-weekly treatment group and 1.3 per cent. in the weekly treatment group had relapses. All these patients knew they had relapses and revisited our clinic voluntarily. There were another 528 patients

TABLE IV.

RELAPSE IN SALAZAR PATIENTS IN WHO *Leishmania* DISAPPEARED ON CESSATION OF TREATMENT.

	Weekly treatment	Twice weekly treatment	Total
Total number of cases	690	395	1,085
Number of relapses	6	11	17
Per cent.	1.3	4.1	2.3
Average period of relapse (in months)	6.0	2.8	3
Time range	1-11	1-2	1-11

This number includes additional cases besides the 783 cases mentioned above. Of this total, about 19 per cent. of patients belonging to the weekly treatment group and 1 per cent. belonging to the twice-weekly group had not completed the six injections as scheduled.

who came back for post-treatment examination. All were in normal health. Sternal puncture was performed in 353 of these, and spleen puncture in 45, all gave a negative result.

Since not all patients complied with our request of follow-up examination, a final check up was made by visiting patients at home *at random*, half to 2 years after conclusion of treatment. Two hundred and seventy-two patients were visited, 131 of whom (44 per cent) never had any previous post-treatment examination. Of this total, nine patients were dead and 263 alive (Table V). All the cases of mortality were due to acute diseases of undetermined aetiology. All the remaining patients were showing normal physical condition and normal growth, and apparently were enjoying good health. There was no case of relapse. It was our impression that if a patient failed to come back for the follow-up examination, he was either dead or entirely cured.

TABLE V
RESULTS OF HOME-VISITS OF PATIENTS 1 TO 2 YEARS AFTER CONCLUSION OF TREATMENT

Group	Average duration of observation (months)	Number of patients visited	Survival		Death	
			Number	Per cent	Number	Per cent
Weekly	11	144	130	90.5	5	3.5
Twice-weekly	10	124	124	99.9	4	3.1

DISCUSSION

In the past, evaluation of drugs for treating kala-azar was chiefly based upon clinical impression. There never has been any simple criterion to determine when a cure is reasonably ensured. Nor has there been any objective means of comparing the condition of patients to whom the drug is administered. The results of our investigation reveal that the number of parasites present in the bone marrow smear can be used as an index of the degree of infection, that their gradual decrease in number parallels the extent and effectiveness of treatment, and that their disappearance from the bone marrow can be used as a simple criterion for cure.

It was further revealed by our investigation that the rate of disappearance of parasites from the bone marrow depended not only on their original numbers prior to treatment but also on the frequency of injections of the drug. It has been clearly demonstrated by us that for a given total dosage of urea stibamine, weekly injections definitely gave better results than twice-weekly injections, evident by the rate of disappearance of parasites, the improvement of the blood picture, and the lower incidence of relapses.

It is evident that the total dosage is determined to some extent by the degree or severity of infection, but it is also influenced by the frequency of administration of the drug. It is not yet thoroughly understood why weekly injections, in the case of urea stibamine, give better result than twice-weekly injections. In treating Chinese hamsters with visceral leishmaniasis with solustibosan, WANG (1939) also found that the cure rate with twice-weekly injections was twice as high as with daily injections. All this suggests that the blood concentration of the drug is not required to be kept at a constant high level all the time. A periodical "boost" seems to be all that is necessary; the rest will be taken care of by the host himself.

SUMMARY

1. Seven hundred and ninety three kala-azar patients in North-west China were treated with urea stibamine during July 1944 and June, 1946, and the period of observation extended to June, 1947. Of this total, 449 patients received the treatment once a week, and 344 twice a week.

2. While the individual dosage was kept constant with regard to the patient's age and body weight, the rate of disappearance of *Leishmanias* from the bone marrow of a patient under treatment was found to depend upon the following two factors:

(a) The number of *Leishmanias* present in bone marrow smear prior to treatment. The more the parasites, the more injections are required to make them disappear.

(b) The frequency of injections with which the drug is administered. For a given total dosage, divided into equal number of doses, weekly injections give better results than twice weekly injections, as shown by the rate of disappearance of parasites from the bone marrow, the improvement of the blood picture, and the lower incidence of relapse.

3. It is recommended that (a) the number of *Leishmanias* present in the bone marrow smear prior to treatment be taken as a simple index of the severity or degree of infection; (b) the rate of their disappearance under therapy be taken as a means to estimate the potency of drugs; and (c) their final disappearance be taken as a simple criterion of cure.

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ACUTE MENINGO-ENCEPHALITIS OF UNCERTAIN ORIGIN IN WEST AFRICAN TROOPS

BY

W M PRIEST, M D, M R C P,*

*Physician, Warneford General Hospital, Leamington,
late Officer-in-charge of a Medical Division, R A M C*

There is a large and growing literature on the neurotropic viruses but little is known about their clinical manifestations in the tropics, and few reports on this subject have appeared. This is a matter of particular interest in that, owing to the occurrence of neuro-syphilis, cerebral malaria, trypanosomiasis and other tropical infections with cerebral involvement, the problem of diagnosis is a formidable one. MUWAZI and TROWELL (1944) reviewed 269 cases of nervous disease admitted to hospital in East Africa over a period of 2 years, remarking that the main causes of "coma" in their hospital are malaria, meningitis, virus encephalitis and "infective coma" (*e.g.*, occurring with pneumonia), these conditions appearing with approximately equal frequency. GELFAND (1944) refers to an acute disseminated encephalo-myelitis which is seen in small outbreaks occurring spontaneously, not related to the exanthemata.

This paper, written mainly from the clinical standpoint, describes cases of acute diseases of the nervous system in West African troops, believed to be due to a virus infection, since circumstances were such that identification of a specific virus was not possible in any, its main purpose is to draw attention to the clinical resemblances within the group (admittedly it may not have been a homogeneous one) with a view to stimulating further observations in this field.

There were confusing factors in almost every case, and it should not be overlooked that the laboratory attached to the hospital at which these cases

* I am indebted to Dr G M FINDLAY, C B E, lately Consulting Physician, West African Command, for permission to publish this paper, and for helpful advice in its preparation, to Major L N GRUNBAUM, R A M C, for the clinical pathology, post-mortem and histological examinations, and to Professor DOROTHY RUSSELL for help in the interpretation of such material as was available for microscopical examination, also to Drs R D HARDING and G SAUNDERS for additional information on trypanosomiasis, to medical officers of No 68 General Hospital, and to others who have helped with advice or additional information. I have to thank Mr J FOREMAN, photographer at University College Hospital, for the micrographs.

were seen was not provided with experimental animals. Although a central laboratory existed, long distances and the vagaries of military transport were a frequent source of disappointment. Where important information is lacking it is necessary to plead the exigencies of field service. Other difficulties arose from ignorance of native languages for example the actual day of onset of symptoms may not have been exact, and the daily assessment of the patient's subjective condition could not be as accurate as was wished. The clinical records, however may be taken as accurate in all important features.

CASE HISTORIES.

(All the patients were serving West African troops mostly from Nigeria, aged 19 to 39).

CASE No 1 Age 36. Admitted 11.3.44, complaining of 2 days severe headache. Drowsy but reasonable. Temperature, 99-4°. Neck rigidity marked Kernig's sign absent. Coarse symmetrical nystagmus. No other abnormal physical signs.

Progress 17.3.44 Drowsy, confused and non-co-operative. Low fever and neck rigidity persist. Convulsion, 19.3.44; Another convulsion. Frequently hiccoughs. Rt. facial weakness of lower neuron type. Left ptosis. Upper abdominal reflexes absent. Knee-jerks weak. Plantars absent. 20.3.44 Left external rectus weakness. 21.3.44 Low drowsy. Squint disappeared. Ptosis marked and right pupil larger than left, both react to light. Nystagmus and neck rigidity persist. 3.4.44 Drowsiness almost disappeared and is alert and sitting up. Rt. facial weakness persists. 21.4.44; Afebrile and convalescent but facial weakness still present. 9.5.44 Now up and about. Bilateral central deafness right complete left partial. 23.6.44 Physical condition good. Facial weakness less marked deafness improving. 5.7.44; Was considered fit for discharge but suddenly had convulsion succeeded by others and died without regaining consciousness.

Laboratory Investigations. In this and all the cases reported, except the blood Kahn reactions referred to later, no relevant laboratory investigations carried out give abnormal results apart from the C.S.F.

C.S.F.	Appearance	Pressure.	Cell	Protein, mg. %	Globulin.
11.3.44	Clear faint yellow	Increased	400 1/3 poly 5/3 haeph.	200	Increased
19.3.44	Faintly turbid pale yellow		1736 mostly haeph 130 R.B.C.	180	—
21.3.44	Faintly turbid, pale yellow		240	160	—
14.4.44	Clear pale yellow	Normal	133	270	Much increased
6.44	Clear colourless		67	80	Negative

A manometer was used throughout this series of cases. Judgement as based on the (admittedly fallacious) nature of the flow.

In this and all other cases all cultures were sterile and no organisms were seen in any specimen. Similarly, the values for sugar and chlorides, where investigated, were all within normal limits. A "—" means that no observation was made.

CASE No 2 Age 32 Admitted 15 3 44, complaining of weakness of left arm and leg and headache for 1 week. Drowsy, but rouseable and co-operative in examination. Complained of pain in the right temporal area, to which he kept pointing. Temperature, 99.8°. Neck rigidity marked, Kernig absent. Complete flaccid paralysis of left arm, partial spastic paresis of left leg. Doubtful left extensor response. Other reflexes normal. No other abnormal signs.

Progress 19 3 44 Right facial weakness of upper neuron type. Incontinent of urine. 21 3 44 Afebrile. Still headache but less drowsy. Facial weakness disappeared. Slight left ptosis, but pupils equal and active. 28 3 44 Much less drowsy and some return of power to left arm and leg. 11 4 44 Again lethargic and incontinent of faeces, but fully rouseable. Left leg weaker again. Left ankle clonus, sustained. Plantars equivocal. 21 4 44 More alert. Left hemiparesis remains. Cranial nerves normal. Plantars flexor. Abdominals absent. Tendon jerks brisker on left. 11 5 44 Now up with residual hemiparesis. Gets about with a stick.

C S F	Appearance	Pressure	Cells	Protein, mg %	Globulin
15 3 44	Clear, colourless	Increased	300 1/3 poly 2/3 lymph	—	—
19 3 44			500 mostly lymph a few R.B.C	—	—
22 3 44	Clear, faint yellow	"	420 all lymphs	120	—
3 4 44	Clear yellow, spontaneous clot	—	—	3,600	Much increased
14 4 44	Clear yellow, spontaneous clot	—	180 lymph 730 R.B.C	240	
20 4 44	(Cisternal fluid) Clear, colourless	Normal	44 lymph	80	
25 4 44	Uniformly blood-stained, yellow after centrifugation		19,440 R.B.C	120	Increased
11 5 44	Clear, colourless		20 lymph	180	
13 7 44			30	80	—

CASE No 3. Age 23 Admitted 15.3.44, semi-conscious no history obtainable. Temperature 101 Restless when disturbed, neck rigidity present, Kernig absent. Bladder distended. N other abnormal signs.

Progress 18.3.44 Still drowsy but rouses to answer simple questions. Neck rigidity persists but does not complain of headache. Afebrile. Knee and ankle jerks absent. Plantar flexor 29.3.44 Appears fully recovered, and in full normal health when seen 8 months later

C.S.F	Appearance	Pressure.	Cells.	Protein, mg	Globulin.
18.3.44	Slightly turbid, faint yellow	Increased	810 lymph 1-40 R.B.C.	70	—
22.3.44	Slightly turbid, faint yellow	?	340 lymph.	70	—
29.3.44	Clear colourless	Normal	2	40	Negative

CASE No 4 Age 20 Admitted 15.3.44 Found lying on the ground unconscious. N other history obtainable. Temperature 102.4 Semi-conscious responding only to painful stimuli. Neck rigidity present Kernig absent. Right divergent squint. Knee jerks absent. Abdominals absent. Doubly incontinent. N other abnormal signs.

Progress 16.3.44 Drowsy and confused could be roused to give his name and number but answered no other questions and non-co-operative in examination. Temperature 99.8 to 100.4 Left hemiparesis, including face, arm spastic and leg flaccid. Plantar flexor Kernig sign on right, doubtful on left. Neck rigidity marked. Squint has disappeared. Vomited once and hiccoughs at intervals. 15.3.44: Conjugate deviation of head and eyes to the right. 17.3.44 Increasing stupor died on 18.3.44

C.S.F	Appearance	Pressure	Cells.	Protein, mg "	Globulin.
12.3.44	Clear colourless	Increased	9 lymph.	70	—
14.3.44			80	70	Slight increase

CASE No 5 Age 28 Discharged from hospital 1 month previously after infective hepatitis lasting 6 weeks found then to have penile chancres (no previous anti-syphilitic treatment) and treated after subsidence of jaundice with bismuth, receiving three injections of 1 c. c. of bismuth metal and remaining well till re-admitted 23.3.44 with history of pains in head, neck and all over body of uncertain duration, presumably few days. Had vomited once. Restless and excitable continual jerky movements of limbs of marionette type and arrhythmic muscular spasms. In short passive intervals, lay inert with conjugate deviation of head and eyes to the right. Temperature normal. Well nourished, faint conjunctival aetna. Frequent hiccough. Coarse symmetrical myasthenia. Owing to the incoherence and lack of co-operation, extremely difficult to examine but no other definite abnormal signs found.

Progress ~ 24 3 44 Vomited again Temperature, 99 6° Conscious, but irregular movements continue, increased on passive movements of the limbs At times lay helpless, eyes rolling, mouth open and dribbling, and sometimes with a fixed expressionless stare Severe dysarthria and dysphagia Incontinent of urine Reflexes all brisk so far as could be ascertained Plantars equivocal, probably flexor No meningitic signs or cranial palsies Later this day became stuporose, rouseable only with painful stimuli Temperature, 102 8° By midnight in deep coma and unrouseable, left eye deviated inwards, 1½ hours later temperature 105 2°, and death occurred in a convulsion

C S F Clear pale yellow, viscous and draining with difficulty, spontaneous clot, in the residual fluid were 13 mononuclear cells and 98 R B C, protein over 100 mg per cent Sterile, no trypanosomes or other organisms seen No further specimen of C S F was obtained except at postmortem, the protein content of which was 220 mg per cent

These five cases were all admitted to hospital within the space of 12 days and recalled two cases previously admitted in which the diagnosis had been doubtful, a form of acute encephalitis of virus origin being considered probable, a description of these follows

CASE No 6 Age 22 Admitted 11 12 43, having fallen down at morning parade and reported "sleepy and confused" on examination by the Unit M O later On admission Temperature 100°, well nourished Drowsy, and described in the admitting officer's notes as "Negativistic, but will respond to sharp commands ? Hysteria" No other abnormal signs

Progress 13 12 43 Temperature, 100° to 101° Drowsy and hiccuping 16 12 43 Still drowsy, head retraction and Kernig's sign present Doubly incontinent Afebrile 19 12 43 Comatose Spastic weakness of left arm and leg Retention of urine Temperature rose suddenly to 105°, died

C S F	Appearance	Cells	Protein mg %	Globulin
13 12 43	Clear colourless	360 R B C	85	—
16 12 43	Uniformly pink and turbid	7 mono R B C + + +	88	—
17 12 43		13 mono R B C + + -	—	—

In this case the clinical diagnosis had been spontaneous subarachnoid haemorrhage from a cerebral aneurysm, but none was found postmortem

CASE No 7 Age 28 Admitted 3 2 44, complaining of dizziness, "eyes turning," pain in the legs and vomiting for 3 days Conscious and rational Temperature, 100 6° Severe vertigo, gait staggering towards the left, coarse nystagmus to the left, head remains turned to the left in bed, hypotonia of left arm and leg, ataxy, dysdiadochokinesis, past-pointing and rebound phenomenon in left arm, dysarthria and staccato speech Right pupil larger than left and barely reacting to light Abdominals absent Knee and ankle jerks reduced but equal Plantars flexor No meningismus and no evidence of ear disease

Progress Pyrexia continued throughout the illness from 100° to 103° The signs of a left cerebellar lesion increased and general condition deteriorated, no headache,

but severe attacks of giddiness even when lying in bed, and frequent vomiting. Nine days after admission sunk into coma, bloodcount increased, and died 14 days after admission.

C.S.F.	Appearance	Pressure	Cells	Protein, mg.	Globulin
4.2.44	Clear colourless	Normal	8 lymph.	70	—
11.11.44	Clear pale yellow spontaneous clot			45	—

CASE No 5 Admitted 26.10.44 found rolling on the ground, semi-conscious, no other history obtainable. Temperature 102°. Comatose neck rigid ty and Kernig's sign present. Left hemiparesis including face arm flaccid, leg spastic. Plantar flexor. Left abdominal reflexes absent. Urinary incontinence.

Progress 2.11.44 Meningismus and drowsiness less marked, temperature falling. Some return of motor power in left leg and arm. 6.11.44 Can answer questions on routine, a quiet peace. 11.11.44 General improvement, but hemiparesis persists. 19.11.44: Temperature rose again to 103° and he subsided into deep coma. 20.11.44 Temperature 105° death.

C.S.F.	Appearance	Cells	Protein, mg. %	Globulin
26.10.44	Clear colourless	130 lymph.	120	Wright curve 4
1.11.44		143	225	—

Review of the first seven cases led to the question whether some of the patients previously diagnosed "clinical trypanosomiasis" on account of changes in the C.S.F., but not showing typical features of this condition, might have had a virus infection of the nervous system. Two such cases had been transferred from another hospital some months after the onset of their illness. The earlier notes on these cases are, unfortunately incomplete but are summarized briefly as follows.

CASE No 9 1 December 1943 had some kind of seizure on road conveyer severe headache and collapsed. Was in sick quarters for 3 weeks before reaching hospital. On admission he was apyrexial and found to have bilateral facial weakness and nerve deafness. No note was made on his mental status other than one by the otologist who examined him, who remarked "At home I would have suspected encephalitis lethargica."

C.S.F. Cells 428 lymph. protein, 200 mg. per cent. No trypanosomes were found.

Seen 8 weeks later he appeared physically well but mentally dull (! due to deafness) he behaved as if stone-deaf, but some bone conduction was present on both sides though accurate investigation of hearing was impossible. He had residual facial nerve paresis of lower neuron type.

C.S.F. Clear fluid and normal pressure. Cells 31 lymph. protein, 80 mg per cent. No trypanosomes.

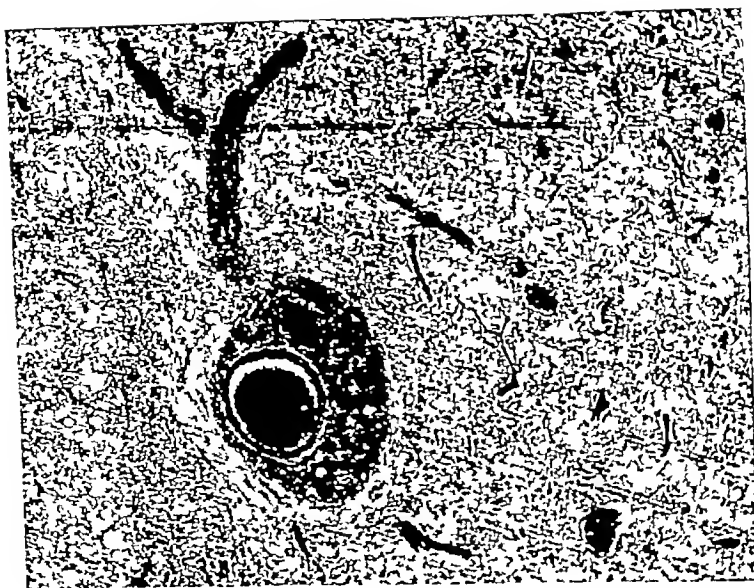


FIG 1 (Case No 8)

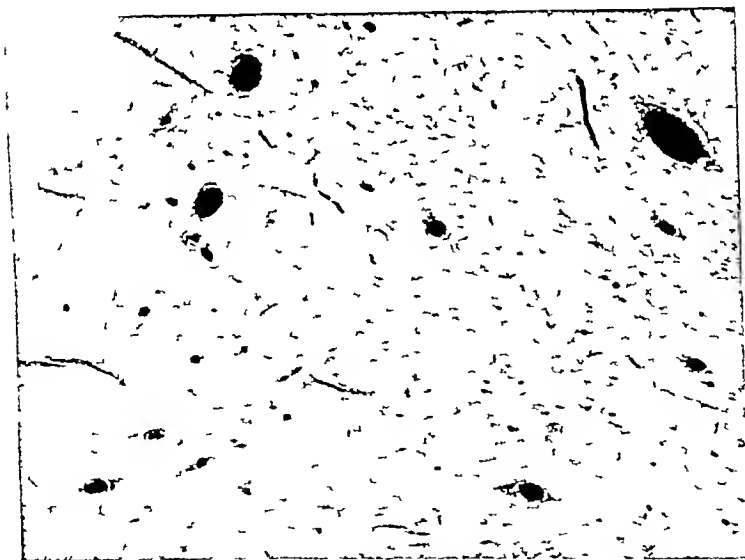


FIG 2 (Case No 4)

FIG 1 shows intense congestion, cuffing and infiltration of brain substance FIG 2 shows intense congestion and slight cuffing

but severe attacks of giddiness even when lying in bed, and frequent vomiting. Nine days after admission sank into coma, hiccough increased, and died 17 days after admission.

C.S.F.	Appearance	Pressure	Cells	Protein, mg. %	Globulin
4.2.44	Clear colourless	Normal	8 lymph.	0	—
11.2.44	Clear pale yellow spontaneous clot			67	—

CASE No 8 Admitted 26.10.44 found rolling on the ground, semi-conscious; no other history obtainable. Temperature 102°. Constant neck rigidity and 'kernig' sign present. Left hemiparesis including face, arm flaccid leg spastic. Plantar flexor. Left abdominal reflexes absent. Urinary incontinence.

Progress 2.11.44 Meningismus and drowsiness less marked, temperature falling. Some return of motor power in left leg and arm. 6.11.44 Can answer questions on routine, aquant present. 11.11.44 General improvement, but hemiparesis persists. 19.11.44 Temperature rose again to 103° and he subsided into deep coma. 20.11.44 Temperature 105° death.

C.S.F.	Appearance	Cell	Protein, mg. %	Globulin
24.10.44	Clear colourless	130 lymph.	120	Slight increase
1.11.44		143	225	—

Review of the first seven cases led to the question whether some of the patients previously diagnosed "clinical trypanosomiasis" on account of changes in the C.S.F. but not showing typical features of this condition, might have had a virus infection of the nervous system. Two such cases had been transferred from another hospital some months after the onset of their illness. The earlier notes on these cases are, unfortunately incomplete but are summarized briefly as follows.

CASE No 9 1 December 1943 had some kind of seizure on road coming severe headache and collapsed. Was in sick quarters for 3 weeks before reaching hospital. On admission he was apyrexial and found to have bilateral facial weakness and nystagmus. No note was made on his mental status other than one by the ophthalmologist who examined him, who remarked "At home I would have suspected encephalitis lethargica."

C.S.F. Cells 428 lymph protein, 200 mg. per cent. No trypanosomes were found.

Seven 9 weeks later he appeared physically well but mentally dull (? due to deafness) he behaved as if stone-deaf but some bone conduction was present on both sides though accurate investigation of hearing was impossible. He had residual facial nerve paresis of lower neuron type.

C.S.F. Clear fluid and normal pressure. Cells 31 lymph.; protein, 60 mg. per cent. No trypanosomes.

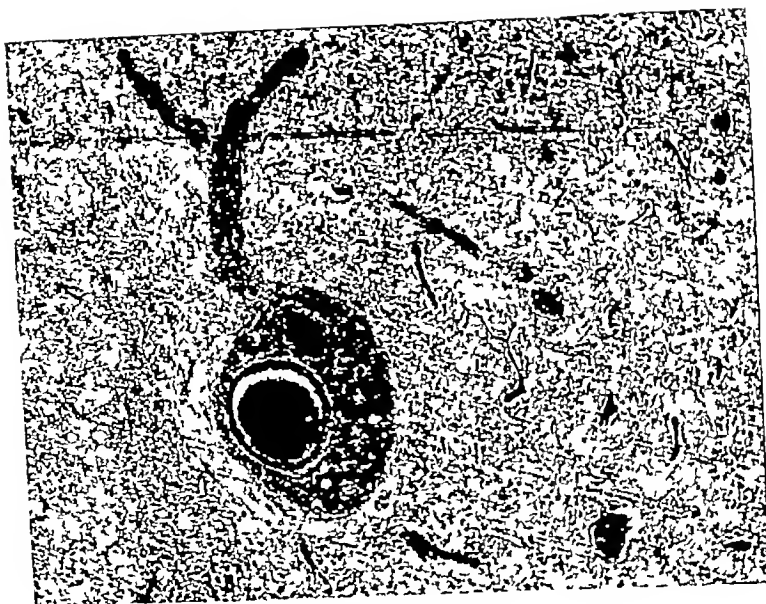


FIG 1 (Case No 8)

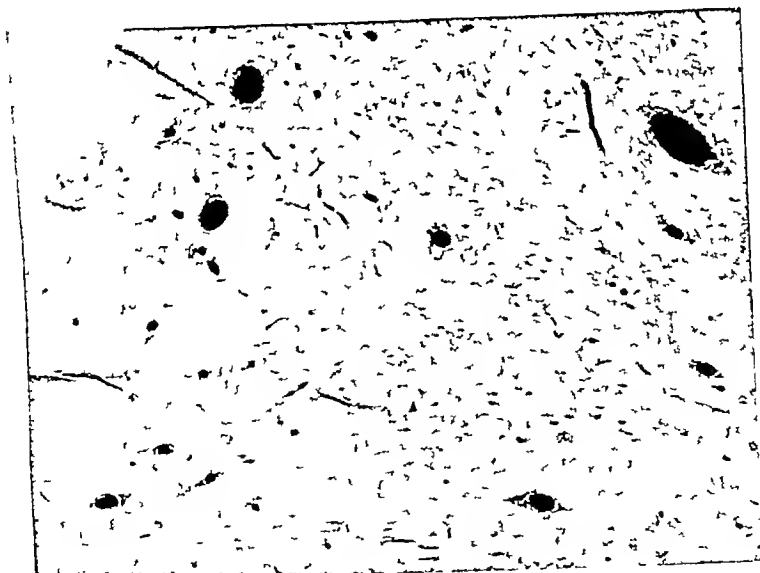


FIG 2 (Case No 4)

FIG 1 shows intense congestion, cuffing and infiltration of brain substance FIG 2 shows intense congestion and slight cuffing

EPIDEMIOLOGY

In attempting to decide whether any or all of these cases constitute an outbreak of an acute specific disease, the following considerations are to be taken into account. Cases Nos. 1 to 5 were admitted to hospital within the space of 12 days, and nine of the ten cases had been taken ill within a period of 3 months. Of the first five four came from the same Details Camp, from which a large proportion of the hospital patients were drawn, and of these, three had been there for periods varying from 3 to 4 weeks, and the fourth for 5 days. The time incidence and sources of the cases is indicated in Fig. 4.

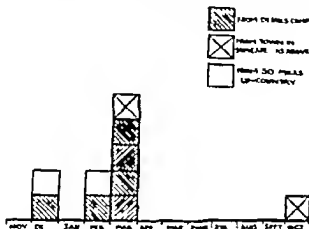


FIG. 4

The possibility that the incidence was other than sporadic was not considered until the end of March when these five cases had been admitted: the two earlier direct admissions in December and February were then recalled, and subsequently attention was directed to the two transfers from up-country who also fell ill in December and February. Although the numbers are small, there is at least the suggestion of a minor "outbreak." The incidence does not suggest case to-case spread, in any event uncommon in virus disease of the nervous system, but rather infection from a common source, possibly by mosquitoes.

Of the two cases which occurred during February one came from the camp in question, and of the two in December 3 months prior to the main "outbreak," one also came from this camp. Sporadic cases of infection with a mosquito-borne neurotropic virus might well be expected: it is pointed out, however, that encephalitis within the colony did not suggest that a virus encephalitis had yet been recognized as of common occurrence, and cases of a

W M PRIEST

CSF															Remarks.
Case number	Day of disease on admission.	Pyrexia maximum height and duration	Headache	Meningismus	Coma	Cranial nerve palsies	Peripheral palsies	Convulsions.	Dysphagia or dysarthria	Nystagmus	Hiccough	Maximum cell count	Maximum protein, mg %	Xanthochromia.	
1	3	90 8 + 4 weeks	+	+	+	R VII L III L VI R & L VIII	—	+	+	+	+	1,730 lymph. 130 R B C	270	+	Died in convulsion on 116th day
2	8	100 5 days	+	+	+	R VII L III	Left hemiplegia	—	—	—	—	500 lymph	3,500 (3 5%)	+	Recovery with residual hemiplegia
3	21	101 4 3 days	?	+	+	—	—	—	—	—	—	510 lymph 1,240 R B C	80	+	Complete recovery
4	21	101 6 5 days	?	++	+	L VII R VI	Left hemiplegia	—	+	—	++	80, 95% lymph	220	+	Died on 3rd day
5	23-4	105 2 3 days	+	—	+	L VI	—	+	+	—	—	13 lymph (clot)	90	—	Died on 7th day
6	1	105 7 days	?	++	+	—	—	—	—	—	++	13 lymph R B C +++	70	+	Died on 16th day
7	4	103 15 days	—	—	Terminal only	R III	(Cerebellar ataxia)	—	+	++	+	8 lymph (clot)	225	—	Died on 25th day
8	21	105 3 weeks	?	++	++	Squint	Left hemiplegia	—	—	—	—	143 lymph	200	—	Recovery with residual deafness
9	23 weeks	+	+	?	?	R & L VII R & L VIII	—	—	—	—	—	428 lymph	110	—	Recovery with residual hemiplegia
10	4	99 6	+	+	+	L VII	Left hemiparesis	—	—	—	—	—	—	—	—

similar type were not being admitted to other hospitals, civil or military, at the time

No definite conclusions as to seasonal incidence, if any, can be drawn, but nine of the ten cases occurred during the months December to March, the hottest and driest time of year

DISCUSSION

The cases reported were all examples of an acute illness with sudden onset, fever, headache, meningismus, cranial palsies, hemiplegia and monoplegia in some instances, coma, and a variable pleocytosis in the spinal fluid, together with a marked rise in protein, six of the ten were fatal. These facts strongly suggest an infection by a neurotropic virus. It remains for discussion whether the same aetiology could have been present throughout the series and to consider alternatives

Trypanosomiasis Since this condition may present some points of resemblance to the cases reported, it will be considered first. Moreover, three of the cases (Nos 4, 9 and 10) had fallen under suspicion of this disease at some time prior to their acute illness. When a man reported sick with drowsiness and was found to have an altered C S F, no trypanosomes being found, the diagnosis of "clinical trypanosomiasis" was usually made. It is suggested that this procedure, while useful in practice, may be fallacious. Certain workers in West Africa are aware of this, and SAUNDERS (personal communication, 1944) remarks that he and some French workers had been sceptical for some time as to whether all cases showing only an abnormal C S F, the organism not having been found, were in fact suffering from sleeping sickness, it was considered, however, the safer procedure to treat them as if they were. The possible occurrence of a virus encephalitis in sleeping sickness areas is thus clearly of importance to workers in this field.

With respect to the cases discussed in this paper, it does not appear that in any can a diagnosis of trypanosomiasis be entertained. In none were trypanosomes found in the spinal fluid (the number of C S F examinations varied from two to 11 in each case), and although this is far from conclusive evidence of their absence, it is unlikely that in no single instance would they have been found. None showed glandular enlargement, all were well nourished, and all had been fit enough for active service with their units until taken suddenly ill.

It is unusual for the protein level in trypanosomiasis to rise much above 120 mg per cent, polymorphs have never been reported and evidence of brain-stem involvement with cranial palsies also appears to be extremely uncommon (R. D. HARDING, personal communication, 1945). The rarity of cranial palsies is confirmed by a search of the literature. VAN DEN BRANDEN and APPELMANS (1934) stated that "diplopia, paralytic strabismus and Argyll-Robertson pupils" had not then been reported and do not refer to other cranial palsies, another authority states that ocular pareses have sometimes been noted but

without unequivocal relationship to the effects of trypanosomiasis (HILSETT, 1930). Hemiplegia or monoplegia is also very rare. One case of hemiplegia occurring in a case of "malignant trypanosomiasis" is reported as a curiosity by FAY (1945). This case had an exceptionally high C.S.F. protein (1.2 per cent.), and trypanosomes were not found—nor did the case react to specific treatment for 2 years. It thus bears some resemblance to those reported in this series. Meningismus is not a common feature of sleeping sickness.

Of the first eight patients the two who survived did not receive any trypanocidal treatment, and in these it is safe to dismiss this diagnosis finally so far as the cause of the acute condition is concerned. Of those who died, the course and clinical picture would seem to make it certain that trypanosomiasis was not the cause of the acute fatal illness—this is not to say that any of the three suspects might not have been infected with it.

Finally the postmortem findings should be considered, but the histological changes found in trypanosomiasis bear a close resemblance to those of encephalitis lethargica (VAN ROOYEN and RIGDEN, 1940), and may be indistinguishable from them (NEAL, 1942)—even ample histological material may not afford a decisive answer.

Neuro-syphilis.

MUWAZI and TROWELL stated that more than 50 per cent. of their neurological cases in E. Africa could be ascribed to syphilis, and that cerebral thrombosis is commonly due to this disease. GELFAND also mentions the frequency of meningo-vascular syphilis in which cranial palsies and leptomeningitis are common—he makes no reference, however, to an acute fulminating form. Next to sleeping sickness, syphilis provides the greatest possibility of diagnostic error in this series where most of the symptoms and signs seen may be observed singly or in combination in acute syphilitic meningitis, and with which the alterations in the spinal fluid are for the most part compatible. The histological picture in the fatal cases, however, did not resemble that of meningo-cerebral syphilis with the possible exception of Case No. 8, where it could not be definitely excluded. In this case the C.S.F. Kahn was negative and the course of the illness, fatal in less than 4 weeks, makes it unlikely that syphilis was the cause. There was no sign of thrombosis or cerebral softening in this or any other fatal case in which a hemiplegia occurred.

Case No. 5 had a primary chancre when in hospital for infective hepatitis 9 weeks before his final illness. In view of the pruritus he received no arsenical, and remained well while receiving bismuth till the sudden onset of the illness from which he died in 7 days, too short a time after the primary infection to be due to acute syphilitic meningitis which, in any case, does not present the clinical picture manifested by this case.

Three other cases had at some stage positive blood Kahn reactions—of these Case No. 3 had a positive C.S.F. Kahn—he, however, made a complete

recovery (with follow-up 8 months later) having received no anti-syphilitic treatment. Cases Nos 1 and 2 showed transient positive C S F Kahns for periods of 12 and 5 weeks respectively, the former died 4 months later and no postmortem evidence of syphilis was seen in the sections of brain examined. In Case No 2 the C S F Kahn was negative at the first two examinations, but was positive during the phase of clinical recovery, remaining so for 5 weeks and reverting to negative without any anti-syphilitic treatment.

A positive Kahn in the blood was so frequent in West African patients, partly owing to the relatively high incidence of yaws and syphilis and partly to transient positive reactions due to such causes as malaria, that it is of little weight as diagnostic evidence in this series. ROBINSON and MCKINNEY (1945), for instance, found positive Kahns in 37 per cent of non-syphilitic patients with *Plasmodium vivax* in the blood, and FINDLAY and ELMES (1945) found 27 per cent positives in British troops with no evidence of syphilis but infected with *P. falciparum*. A positive Kahn in the C S F cannot so easily be dismissed. There are, however, a number of conditions in which a false positive W R may occur in the absence of syphilis, it may be due to the presence of blood in the C S F, this being the case in all the patients in this series in which a positive Kahn was found, furthermore, transient positive Wassermann reactions have been reported in encephalitis (HALL, 1924), and false positives in blood and C S F by KALZ and others (1946) in meningitis of bacterial or virus origin. NEAL (1942) remarks that in Europeans the main differential point between an epidemic encephalitis and cerebro-spinal syphilis would be the C S F Wassermann, but "even this is not entirely reliable unless repeated several times owing to transient false positives".

Cerebral Malaria

This was rare in African troops, no unequivocal case was admitted to the military hospital in question in 18 months. All the patients in this series were given parenteral quinine or mepacrine without therapeutic effect. Features such as meningismus, hemiplegia, cranial palsies and alterations in the C S F may occur individually in cerebral malaria, but in combination are practically unknown. Parasites were not found in life nor in the brain at postmortem. It is of interest that MUWAZI and TROWELL state that many cases of virus encephalitis are incorrectly diagnosed as cerebral malaria.

Tuberculous Meningitis

No evidence of tuberculosis was found in the meninges or elsewhere in any of the fatal cases.

Meningo-encephalitis Due to a Virus

It is considered that the evidence so far adduced, mostly of a negative character, points to the likelihood that most, if not all of these cases, were

examples of infection with a neurotropic virus though no claim is made that the same agent was at work in every case. The isolation of a virus or the discovery of immune bodies to such a virus was, unfortunately, not achieved, and in some could not be attempted. Serum from four cases sent for neutralization tests were all negative for known viruses. Brain substance from Case No. 5 was inoculated intra-cerebrally into mice also with negative results. Circumstances were not favourable for quick transmission to a laboratory where investigations for viruses could be carried out in one case, however brain and cord were inoculated into monkeys, mice and guinea pigs on the same day again the tests were negative. These results do not necessarily exclude a virus infection.

It is difficult or impossible to diagnose the various neurotropic infections on the basis of clinical findings because of their great similarity (MILNER, 1943; REDMAN, 1944). "Benign" lymphocytic meningitis, for example, may be protean in its manifestations and its causative virus may produce a fatal encephalitis (VICTA and WARREN, 1937; HOWARD, 1940; REDMAN, 1944). Case No. 3 in this series appeared to conform in most respects to the typical form of benign chorio-meningitis but on the foregoing considerations it cannot be isolated from the others. Moreover chorio-meningitis may be clinically indistinguishable from the non-paralytic form of polio-myelitis. There had been no known cases of the latter disease notified from military sources for a period of months before and after these cases were seen only seven cases were recorded from 1940 to 1945 out of about 150,000 troops at risk (FINDLAY, ANDERSON and HIGGIE, 1946). It is a possibility however that some of the cases might have been examples of a brain-stem, and in one case a cerebellar form. Nor are the cellular and chemical changes in the C.S.F. of any particular value in identifying a specific virus disease, as the widest range of variation is shown. Some of the cases described bore a more general resemblance to equine encephalo-myelitis than to St. Louis or Japanese B encephalitis they were not characteristic of lethargic encephalitis in any of the varieties generally described.

None of the patients had been recently vaccinated

Certain special features in this series are worth noting. *Restlessness and spontaneous movements* were marked feature in Case No. 5 among *cranial palsies* which occurred in eight out of the ten, involvement of the eighth nerve is very rare in encephalitis of any type (NEAL, 1942) it was seen in two of this series. *Cerebellar syndromes* of which Case No. 7 was an almost pure example, is also uncommon though *crigo* and Benedikt's syndrome has been reported (WACHSLE, 1935). *Fatal complications* have been reported as occurring several months after the onset of the acute disease as in Case No. 1 (NEAL). *Hemiplegia* and *monoplegia* occur in various forms of encephalitis, notably in the St. Louis and Japanese B forms (NEAL, 1942; ALPERS, 1946). ST. WALK and THORWELL also refer to hemiplegic forms of encephalitis of doubtful aetiology. *Hiccough* has been noted in a number of outbreaks and was present in four of this series. *Fluctuation in the intensity of signs and symptoms*, particularly in depth of coma or degree of ocular paresis, for example is very characteristic of this group of diseases and was well marked in the cases here reported.

Cerebro-spinal Fluid

With the possible exception of equine encephalo-myelitis, where the cell count is usually over 1,000, and Economo's disease where it is usually less than 50, the level of the cell-count is of no diagnostic value. Red blood cells were present in Cases Nos 3 and 6 at the onset, in Case No 2 blood was present in considerable quantity during the seventh week and in one other case. In three out of the four, there was xanthochromia. Various forms of haemorrhagic encephalitis have been noted in the past (GREENFIELD, 1933, HOWARD, 1940, NEAL, 1942, MARGULIS *et al*, 1944). Also of interest were the protein levels in the C S F, spontaneous clotting occurred in two and in one of these the fluid was so viscous at the first puncture as to drain only with difficulty. In Case No 2 the protein level reached the exceptionally high figure of 3.5 per cent. This specimen of fluid was not bloodstained and a slight increase of flow was produced on jugular compression, 17 days later, when lumbar fluid could not be obtained, cisternal fluid contained 80 mg per cent only, 21 days later fluid could again be obtained at lumbar puncture and contained 120 mg per cent. A transient spinal block must therefore have been present. In this connection, BARKER and FORD (1937) have reported a case of partial paraplegia occurring in proved lymphocytic chorio-meningitis where no fluid could be obtained on repeated puncture, on surgical exploration a chronic arachnoiditis with obliteration of the subarachnoid space was found.

Incidence of Encephalitis in the Tropics

Apart from the remarks of MUWAZI and TROWELL in their review of neurological cases and GELFAND'S brief reference already quoted, little attention appears to have been devoted to this subject from the clinical aspect. The first-named authors saw 11 cases of "virus encephalitis" with seven deaths in their 269 neurological cases in 2 years, nearly all were admitted stuporose and neck rigidity, coarse tremors, rigidity, nystagmus and pupillary abnormalities were seen but no oculomotor palsies, death occurred in 3 to 4 weeks, and there were no complete recoveries. CHATTERJI *et al* (1945) describe 89 cases of "encephalitis" occurring in Calcutta hospitals in 2 years in which an acute onset with early stupor, fever and often convulsions were features, meningismus and cranial palsies were frequent and the mortality was 72 per cent, reports on the C S F merely say that the "protein was increased".

The existence of neurotropic viruses in the tropics is now increasingly well known. HAMMON and REEVES (1945) state that the evidence is accumulating that there are a large number of such viruses of man and other animals (arthropod-borne) of "world-wide distribution and no small importance". Search for immune bodies to West Nile virus has revealed their presence in the sera of natives over wide areas of Central Africa. Positive results have been obtained in from 2.7 per cent to 45 per cent, of sera tested in different areas,

the higher figure from one district in the Congo, which is the nearest area to British West Africa the survey has so far reached (SMITHURST and JACOBS, 1942). Others such as that named "Semliki Forest" (SMITHURST and HADDOW 1944) have been identified, but little is known of the diseases caused by these viruses in man. It is clear that further clinical observations are required in the search for cases of these allied diseases and that a highly critical attitude should be adopted in the diagnosis of such conditions as cerebral malaria, trypanosomiasis and neuro-syphilis which may so closely resemble the virus infections.

This paper throws no light on the incidence of any particular virus—it is mainly concerned with clinical observations and will have served its purpose if closer attention is directed towards this group of diseases, particularly in the tropics.

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THE TREATMENT OF AMOEBIC DYSENTERY IN THE BANTU AFRICAN *

BY

T G ARMSTRONG, M D, M R C P,
A J WILMOT, B M, B CH

AND

R ELSDON-DEW, M D
King Edward VIII Hospital, Durban

In Natal, and particularly in Durban, amoebic ulceration of the colon is an extremely common and intractable disease. As has been pointed out by one of us (ELSDON-DEW, 1945), it affects predominantly the native population and occurs rather uncommonly in Indians and, to only a small extent, in Europeans. The native in Durban suffers not only more frequently but also more severely than the European. The clinical picture is most frequently that of an acute dysentery closely simulating the bacillary type. Often the history is only a few days in duration though sometimes it may be a few weeks or even months. Macroscopically, the stools tend to consist only of blood and mucus. In a previous paper one of us (ELSDON-DEW, 1946) has discussed the microscopic appearances of the stool, which are those of an acute bacillary dysentery but differ only in the presence of innumerable, actively-motile, haematophagous amoebae. In this paper report was made of cultural results, which failed to reveal any consistent infection with organisms of the dysentery group.

*We have to acknowledge the assistance of Dr D N RANKING with the early groups of the series, and have to thank Dr J C THOMAS for suggestions. We would like to place on record our appreciation of the services of Mr GOODCHILD, male nurse in charge of the special Amoebic Dysentery Ward, so kindly placed at our disposal by the Superintendent of the hospital, Dr J L PARKER.

Constitutional disturbance is only moderate and most patients are ambulant although there may be much loss of weight. Malnourishment and cachexia are, on the whole, not common and we have seldom seen gross vitamin deficiencies in our cases of amoebiasis. A certain proportion of cases are admitted in *extremis* with perforations and general peritonitis, and it is indeed remarkable how quickly this catastrophic condition can develop. In such cases the colon is occasionally as thin as paper with multiple perforations in its attenuated wall. More often, perhaps, there is much thickening of the bowel wall with granulomatous proliferation. In such cases the wall is friable and oedematous like wet blotting paper.

Sigmoidoscopically the bowel may show a considerable variety of lesions. It is rather uncommon to find the classical picture of shallow angulated ulcers set in a normal mucosa. The whole membrane is more often pink and oedematous, while often it is uniformly and acutely inflamed and presents a crimson velvety appearance. Ulcers vary from the shallow angular variety a few millimetres in diameter to indurative lesions the size of a half-crown. A relatively common finding is a tough leathery induration in and around the ulcer which, in extreme cases, may progress to amoeboma formation and even stricture. In nearly all cases the process has been much more marked in the rectum particularly in its lower part, and the sigmoid colon has been either relatively free of ulcers or normal.

Because of the extraordinary numbers of cases at King Edward VIII Hospital and their poor response to treatment, we decided that we would attempt an assessment of the comparative values of the various modern methods of treatment using them singly and in combination. The object of this work was to test in a large series of cases the effect of various drugs in bringing an attack of amoebic ulceration under immediate control, as judged by a remission of symptoms, disappearance of amoebae and healing of ulcers.

We wished to find the best and quickest way of terminating the acute attack and we wished to learn on which drugs we could rely and which, if any, could be profitably discarded. We also desired accurate information on the failure rate of treatment in the African. It has proved quite impossible to carry out any follow up investigations. Our figures therefore relate only to what one may call immediate apparent cure and give no information on the subject of permanent or radical cure. The relapse rate is therefore unknown and until the education of the African improves and the probability of re-infection is reduced, it is likely to remain so.

The work has been of an experimental nature and designed primarily to give information on the value of certain drugs rather than at the outset to provide complete or permanent cure or indeed freedom from relapse. Some efficiency in the treatment of the earlier patients has therefore knowingly been sacrificed.

In this work certain criteria of diagnosis were needed and were rigidly

adhered to. In our series we accepted only those patients in whose bowels ulcers were seen with the sigmoidoscope and from whom actively motile amoebae were observed under the microscope. No patient in whom only cysts were found has been included in any series. Before treatment of any kind was started records were made of the sigmoidoscopic appearances, and this was repeated during the course of treatment and always at the end. The final sigmoidoscopy was done at 20 days from the commencement of treatment. The final result was recorded under the following headings:

(1) *Success*—The mucosa was normal or at the most showed some pinkness and oedema obscuring the vascular pattern. All ulcers had soundly healed.

(2) *Doubtful success*—The remains of ulcers were clearly visible, but were completely epithelialized though still hyperaemic.

(3) *Failure, but with no amoebae*—Open ulcers were present but scrapings and purged stools failed to show amoebae even by Faust's flotation technique which has been so successful in our hands (ELSDON-DEW, 1947).

(4) *Failures with amoebae*—Open ulcers were visible and amoebae were demonstrated.

Groups 3 and 4, that is all cases in which ulcers were seen after treatment, may be considered as failures, though Group 4 are the absolute failures and are of most importance. Groups 1 and 2 might well be combined, in that their differentiation is sometimes surprisingly difficult and liable to personal error. For purposes of discussion this combination will be referred to as probable success.

We started by comparing the effects of two drugs given separately to two different series of patients. One series of 50 cases received one grain of emetine nightly for 15 nights, the second series received 10 tablets of diodoquin daily for 20 days. The results were as shown in the table and chart. The two series were then examined statistically and it became clear that no significant difference existed between the immediate results of emetine and diodoquin. These are most surprising results. For years emetine has been the sheet anchor of the immediate treatment of the symptoms of amoebiasis. We consider that confidence in the value of emetine has been in no way misplaced. Diodoquin is a relatively new drug and, though widely used, has seldom been subjected to critical analysis on a large scale. Our figures show that diodoquin is as powerful an amoebicide as emetine. But the figures also underline the very serious shortcomings of each, at least in the treatment of the local African. We do not believe that these shortcomings are in any way so important in Europeans. However, no drug can be accepted with equanimity as a specific, when its failure rate approximates the high figure of 50 per cent.

In contrast with the effect of emetine and carbarsone in the native, one of us (ARMSTRONG) during the late war in M E F, treated 11 consecutive patients with emetine grain 15, a course of carbarsone and a course of retention enemata of yatren. No E B I was used. All patients were cured on discharge and all sigmoidoscopies were normal. On follow up at 6 months, ten replied that

they had had no relapse. One had had a proved relapse. At 12 months, seven replied that they had had no relapse and three did not reply. We consider that the satisfactory nature of these figures shows that Europeans respond to emetine much more rapidly and completely than the African under local conditions.

Our next step was to combine the two drugs. Emetine was given for 15 days concurrently with diodoquin for 20 days. The differences shown in the table are statistically significant and there is, of course, no doubt of the

TABLE.
ULCERATIVE AMOEBIAC COLITIS: TESTS OF THERAPY

Drug	Number of cases.	Results per cent.			
		Success.	Doubtful success.	Failure no amoebae.	Failure with amoebae.
Emetine gr 10	44	28	14	23	35
Diodoquin	50	22	16	18	44
Emetine gr 15 and diodoquin	34	33	1	36	
Emetine gr 15 diodoquin and hydrochloric acid	48	6	13	1	
Emetine gr 10 diodoquin	62	4	17	23	56
Emetine gr 10 carbarsone	48	27	31	31	11
E.R.L., diodoquin	50	42	36	14	8
Emetine gr 10 diodoquin, gentian violet	53	13	8	13	66
Penicillin sulphasuxidine, emetine gr 10 diodoquin	39	64	31	5	0

greatly increased value of the two drugs used concurrently. Most significant is the change from 28 and 24 per cent. of amoebic infected ulcers for emetine and diodoquin alone to 2 per cent. when the two drugs were combined.

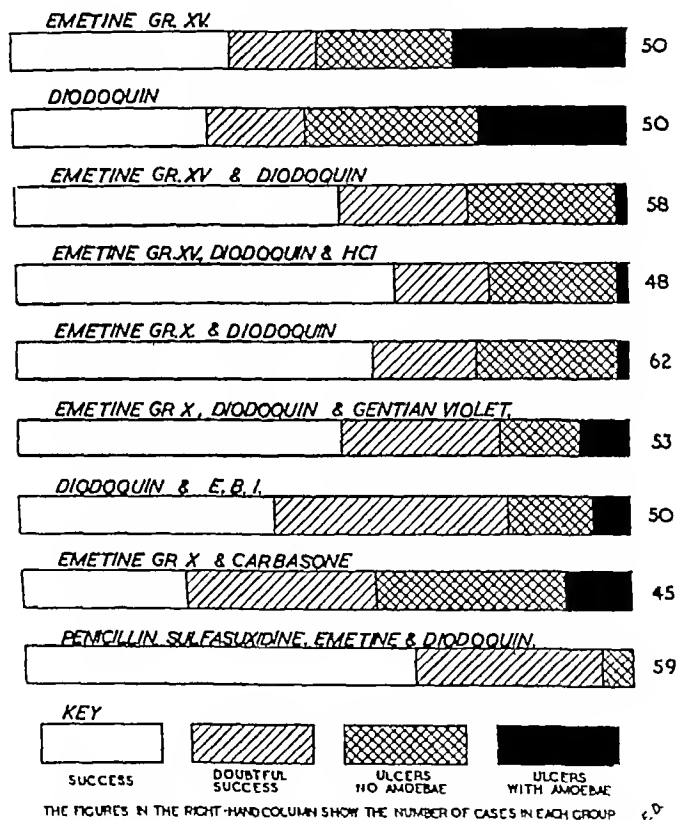
We then treated 60 patients with the standard course of diodoquin but gave them emetine grain 10 only. When subjected to analysis, the results differed insignificantly from the original course employing five more grains (15 in all) of emetine. We concluded that 10 grains of emetine were as effective as 15.

A course of emetine grain 10 was then given in conjunction with a 10-day course of carbarsone to 45 patients. This was the standard treatment previously used in the hospital. Failures totalled 42 per cent., of which 11 per cent. still showed actively motile amoebae. The probable success rate was 58 per cent. Comparing these results with the series in which diodoquin was combined with emetine grain 10 it is clear that carbarsone is a much weaker amoebicide than

diodoquin With diodoquin only 17 per cent showed amoebae while with carbarsone the figure was 11 per cent None the less, analysis and comparison of the figures for emetine and carbarsone on the one hand, and emetine alone on the other, show that the effect of carbarsone is "probably significant" The figures indicate a *probable* therapeutic value but one which is clearly far less than that of diodoquin

ULCERATIVE AMOEBIASIS

TESTS OF THERAPY



Our next series was a trial of emetine bismuth iodide, grain 3, for 10 days with diodoquin for 20 days Fifty cases were treated in this way and the results were as follows Ulcers with amoebae, 6 per cent , ulcers without amoebae, 14 per cent , doubtful success, 38 per cent , success, 42 per cent Statistical analysis shows that there is no material difference between the effects of emetine and emetine bismuth iodide when used in conjunction with diodoquin

(5) There is a suggestion that penicillin and sulphasuxidine alone may have amoebicidal powers, possibly having an indirect action through some organism symbiotic to the amoeba.

(6) These remarks apply only to the immediate cure rate in the Bantu of Natal. No information on relapse rate is available.

SUMMARY

Nine groups, each of approximately 50 cases, of amoebic ulceration of the colon selected under rigid criteria were submitted to various forms of therapy with a view to selecting those drugs giving the most effective immediate cure. Combination of penicillin, sulphasuxidine emetine and diodoquin has given the best results to date.

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AETIOLOGICAL AND PROGNOSTIC FEATURES IN TROPICAL SPRUE

A STUDY OF 47 CASES OVER A 2½-3 YEAR PERIOD *

BY

A W WOODRUFF, MD, MRCP, DTM & H,

*First Assistant, Hospital for Tropical Diseases, University College Hospital, London ,
Lecturer in Clinical Tropical Medicine, London School of Hygiene and Tropical Medicine*

The clinical features of tropical sprue as it occurred among troops in India during the second world war have been described in detail (KEELE and BOUND, 1946, and KEELE, 1946), but no studies of the prognosis of these cases have as yet been reported. Indeed, the literature on this subject is remarkably scanty, during the last 20 years only FAIRLEY (1936) has discussed the subject in any detail. He states, however, that statistical evidence regarding the prognosis in sprue is lacking. KEELE and BOUND (1946) quote ELDER, who for 1 year followed 40 cases of sprue originating among troops in India during the late war. Of these six had had severe relapses during this period and the remainder less severe recurrences of symptoms, none had been quite well.

The only remaining report on the prognosis of tropical sprue published during the last 20 years is that of RODRIGUEZ-MOLINA (1943) and is more favourable. Working in Puerto Rico, he found that of 75 patients discharged from hospital as improved, 65 remained well for 5 years without treatment.

* I wish to thank Dr F MURGATROYD for his criticism and advice, Dr J MARTIN for the statistical comments, the D G M S, Royal Air Force, for permission to publish this paper, and Dr C C UNGLEY for advice regarding the follow-up of the patients.

I am grateful to the University of Durham, Department of Medicine, for bearing the expenses of this investigation, which was mostly carried out while working on the University Medical Professorial Unit.

Before the introduction of modern treatment, Low (1928) followed 150 cases over the period of 20 years, from 1908 to 1928. He found that the average duration of the patients' stay in hospital in England after invaliding from overseas was 29 weeks. During the 20-year period, 10 out of the 150 died, 22 were completely cured, the condition of 60 was described as satisfactory, 22 were improved, 16 were not improved, and 20 could not be traced.

It seemed therefore, that the prognosis in cases of tropical sprue occurring among Service personnel in India and Burma was a matter for further study and in studying their aetiological features of some interest became apparent. The patients are now widely scattered throughout the British Isles, Canada and the U.S.A., making it impossible to examine each personally. A detailed questionnaire was therefore posted both to the patient and to his present doctor. It is from an analysis of these questionnaires that the report on the present condition of the patients is based.

ÆTIOLOGICAL FEATURES IN THE CASES STUDIED.

The patients were admitted to an R.A.F. mobile field hospital serving all Air Force personnel in the Chittagong district during the period July 1944 to June, 1945 and in a large portion of South Burma from June to August, 1945.

Locality. Of the total of 47 cases, 37 contracted the disease in the Chittagong district of Eastern Bengal, and 10 in Southern Burma. Around Chittagong the disease, as has been stated by KEPLER and BOURN (1946), and by LEISDALE (1945) was particularly prevalent. The occurrence of groups of cases in isolated units suggested that an infective agent may possibly have played some part in their causation. Thus, of 11 cases occurring in July 1944 three were from a unit comprising less than a tenth of the R.A.F. population served by the hospital. This unit was housed in a single building—a converted convent. Though such figures could occur by chance once in 10 times, the presence of an infective agent might well explain them.

Season. Fig. 1 shows the monthly incidence of the onset of symptoms of sprue as distinct from the dates of admission in the series. These have been compared with those of 72 cases of amoebiasis. Only fresh cases of amoebiasis proved by finding vegetative forms microscopically in the stool have been used and relapses have been excluded from the series. The months in which symptoms of amoebiasis developed have again been used in preference to the months of admission. This is because of the considerable period which elapses in many cases between onset of symptoms and admission to hospital.

It is seen that the peak incidence of sprue and of amoebiasis occurs at the same time. Although in a small series this cannot be taken as a statistically significant observation, it strongly suggests that the peak incidence of the two diseases tend to coincide. If the incidence of amoebiasis can be taken as an index of the prevalence of alimentary infection it would seem that sprue occurs

at a time when such infection is commonest, and it may well be that an upset in the bacteriological flora of the intestine is a factor in the causation of the disease. This argument admittedly presupposes that amoebic dysentery and sprue have similar incubation periods and of this there is, of course, no certain knowledge. It is stressed that these figures of the incidence of amocbiasis are used only as an index of the times at which alimentary infection is likely to occur. It is not suggested that amoebic infection predisposes to sprue and, as will be shown later, the evidence is against such a suggestion. KEELF (1946) found the peak incidence of sprue to precede that of dysentery. His figures were taken from the admission dates of cases, however, and did not refer to any specific outbreak but were pooled from the whole of India and Burma.

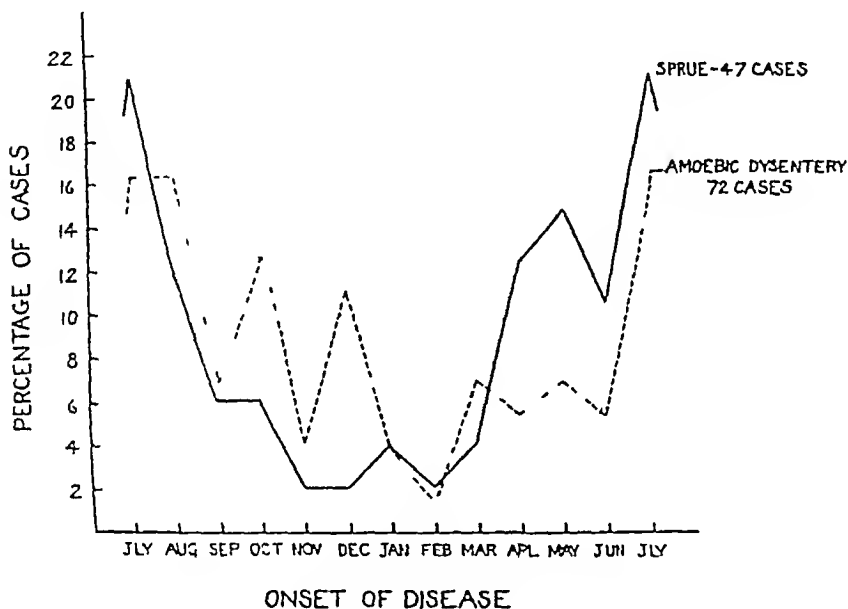


FIG 1—Monthly incidence of tropical sprue, 47 cases, and amoebic dysentery, 72 cases

Tropical Service The average duration of service in India and Burma before admission was 2 years and 2 months, the shortest period was 9 months and the longest 16 years, this latter patient had been a civilian in Eastern Bengal prior to the war. Table I shows the incidence of cases following varying lengths of tropical residence.

It will be noted that the greatest incidence in this series occurred towards the end of the first year of service in India or Burma. It is not suggested, however, that sprue is commonest after this period of tropical residence as it is probable that the greatest numbers of R A F personnel in India at the time were of those who had served in the country for 6 to 12 months.

TABLE I.

DURATION OF SERVICE IN TROPICS PRIOR TO ADMISSION TO HOSPITAL FOR SPRUE
—47 PATIENTS.

Under 6 months. Per cent.	6 months to 1 year. Per cent.	1 to 1½ years. Per cent.	1½ to 2 years. Per cent.	2 to 2½ years. Per cent.	½ to 3 years. Per cent.	Over 3 years. Per cent.
0	47	16	16.2	19.6	19	8.4

Previous Illnesses The previous history of illnesses was ascertained in all the cases of sprue and compared with that in 100 cases of diseases other than sprue or dysentery (Table II). Particular care was taken to ensure that the duration of service in the tropics prior to admission in this control group was precisely similar to that in the cases in the sprue series as shown in Table I.

TABLE II.

PREVIOUS ILLNESSES IN TROPICAL SPRUE, 47 CASES, AND IN A CONTROL SERIES OF 100 PERSONS.

	Sprue series. Per cent.	Control series. Per cent.
Previous history of no illness obtained	34.30	37.0
enteritis or dysentery	23.40	40.0
dysentery	13.01	13.0
amoebic dysentery	4.31	3.0
bacillary dysentery	13.01	10.0
— malaria	13.01	26.0

N.B.—Patients having previous history of more than one of the diseases cited are counted in the figures given against both or all the diseases from which they had suffered.

It will be noted that the previous histories of the sprue patients regarding dysenteric diseases are not significantly different from those of the control group. From this it might be deduced that whether an alteration in bacterial flora of the bowel plays a part in the causation of sprue or not, infection with the organisms producing frank dysentery and enteritis apparently does not predispose to the development of sprue.

CLINICAL FEATURES.

The clinical features in the cases were not exceptional.

Diarrhoea was encountered in every case; the mean duration of this symptom prior to admission being 3.73 months (standard deviation 2.62 months). The average number of motions passed during 24 hours was five.

Tongue Lesions Of the 47 cases, 39 showed well developed tongue signs, of these 20 had glossitis with markedly inflamed and atrophic mucosa and 19 active ulceration. Eight had no tongue symptoms.

Weight loss was invariable, the average being a drop of 28 lb in the course of 2 to 4 months prior to admission.

The appetite was inconstant, two-thirds of the patients had anorexia, the remainder complained of no alteration in the appetite.

Vomiting was complained of by nine (i.e., 19.3 per cent) of the patients, and nausea without vomiting by a further five (i.e., 10.6 per cent).

Abdominal distension was invariably complained of, in 20 cases it was especially severe. Along with distension flatulence was almost always present.

Cramplike feelings in limbs were recorded in 10 cases.

Sigmoidoscopy revealed no characteristic appearances, in the patients with marked anaemia pallor of the mucosa was present. In two other cases injection without ulceration was observed.

LABORATORY FINDINGS

Anaemia was encountered in all but two of the cases. It was seldom of marked degree, the average haematological values for the series were: Red cell count, 3,941,250 (range 2.48 to 5.03 millions). Haemoglobin (Haldane), 81.7 per cent (range 58 to 103 per cent). Colour index, 1.06 (range 0.89 to 1.34). The colour index was unity or above in all but five cases. These findings are in accordance with those of FAIRLEY, MACKIE and BILLIMORIA (1929), who found "normal or only slightly reduced red cell and haemoglobin values in 11 out of 16 cases examined shortly after the onset of symptoms."

Faecal fat was raised in two-thirds of the cases in which this estimation was done, owing to field conditions this was possible in 22 cases only. In these the faecal fat expressed as a percentage of the faecal dried weight averaged 37.12 (range 7.2 to 14.8). The split fat similarly expressed averaged 28.64 (range 6.1 to 13.1). Of the total fat in the faeces, therefore, an average of 77.15 per cent was split. HOWELL (1947) has drawn attention to the large proportion of the faecal fat which is split in early cases of sprue, and these figures appear to support this observation.

TREATMENT AND IMMEDIATE PROGNOSIS

The patients were all placed on a diet high in protein and low in carbohydrate and fat as recommended by FAIRLEY (1930, 1932), in addition supplements of nicotinic acid mg 100 and ascorbic acid mg 50 were added, and whenever possible marmite or vegemite (an Indian yeast product akin to marmite) was given in doses of drachm 1 twice daily. Those cases showing anaemia with less than 70 per cent haemoglobin received parenteral liver extract c.c. 2 thrice weekly. On this regime improvement was invariable and often striking. All patients except one were invalided to United Kingdom after an average stay in hospital of 35 days.

The patients were repatriated by sea, the average duration of the journey from leaving the hospital till arriving in the United Kingdom was 7.7 weeks. An improvement in the symptoms during the journey was experienced in 53 per cent of the 34 patients followed up, a further 20 per cent considered that their condition deteriorated during the voyage, and 27 per cent reported no change. On arrival in the United Kingdom the patients were reviewed in hospital and 57 per cent detained for periods of more than 48 hours, the average stay being 7 weeks; this compares favourably with the 29 weeks needed by

Low's patients prior to 1923. After sick leave they reported for duty the average time elapsing between leaving hospital in India or Burma and reporting for duty was 20.5 weeks.

Condition of Patients 2½ to 3 years later

Capacity for work. All of the 34 patients whom it was possible to contact reported that they were able to carry on their full work. Their employment varied widely—seven were classified as doing heavy manual labour, 14 moderately heavy work, 12 had sedentary posts and the employment of one was unknown.

Weight. An increase in weight during the 3-year period of follow-up is most striking feature in 29 (i.e., 85.2 per cent.) of the patients. The remaining five have not gained or lost more than 2 to 3 lb. The average increase per patient in the series is 24 lb.

Diarrhoea. All the patients stated that they were now free from this symptom, though 67.6 per cent. had complained of diarrhoea at some time since returning to the United Kingdom. The vast majority of the attacks, however, had been infrequent and were of very transitory nature. See Table III.

TABLE III.

FREQUENCY OF DIARRHOEA IN 47 PATIENTS WITH TROPICAL SPRUE FOLLOWING RETURN TO TEMPERATE CLIMATE.

		Number of patients.	Percentage of cases.
N. diarrhoea		11	23.4
Diarrhoea for first 6 weeks after return to U.K. without recurrence	—	4	11.0
Diarrhoea for first 18 months after return to U.K. without recurrence	—	1	2.0
One attack diarrhoea lasting 48 hours since return to U.K.	48	4	11.0
Two attacks	48	3	8.0
Four	48	1	2.0
+	48	4	11.0
One attack	2 to 4 days since return to U.K.	2	5.0
Four attacks	2 to 4	1	2.0
One attack	7		2.0
	14	1	2.0

Glossal Ulceration. Eleven patients (i.e., 32.0 per cent.) complained of ulcers on the tongue at some time since recovery from the initial symptoms. Of these one-half had had an ulcer on three occasions or less, two on four to six occasions and in the remaining three cases ulceration had been more frequent, occurring at intervals of 3 to 5 months over the 3-year period.

Appetite. Only three patients (i.e., 8.8 per cent.) complained that their appetite had not returned to normal although continued disinclination for fatty food was not uncommon.

Abdominal Discomfort and Distension. These symptoms were described occurring frequently by three patients, further six (i.e., 26.4 per cent.) had had an occasional attack usually precipitated by eating fatty foods or taking an excess of alcohol.

Abdominal Pain was recorded in 13 cases (i.e., 33 per cent.). In none of these, however, was the pain more than an occasional spasm after heavy meal or before defecating.

Cramps in the limbs were not encountered in association with diarrhoea or other symptoms in any of the patients

Lazitude and Lack of Energy Only three patients (3, 8.8 per cent) considered that their energy was still significantly impaired, and in no case was this of sufficient degree to interfere with their full employment

DISCUSSION

On surveying the cases as a whole it became apparent that 3 years after contracting the disease all but three patients were very well, and that even these three were not affected severely enough to interfere with their work. In the remaining 91.2 per cent the condition was highly satisfactory, more than a third had no complaint of any kind, and the remainder have had only a very occasional minor and transient symptom. KEELE and BOUND (1946) stated: "Prognosis as regards function on returning to England is not known, but of 40 cases followed up for 1 year, six have had severe relapses, and the remainder less severe recurrence of symptoms. None have been quite well."

This information is depressing. It is hoped that a fuller survey will be less so. It is considered that this more protracted survey is much less depressing and supports FAIRLEY (1939) in his statement: "Granted the co-operation of the patient and the absence of intercurrent disease, patients with tropical sprue, both in the primary attack and in relapses, are invariably capable of being restored to health by appropriate treatment." It is perhaps noteworthy that one of the three patients not completely cured was known to take an excessive quantity of alcohol.

The prognosis in this series appears to be better than in that of LOW (1928), and it is considered likely that this is due to advances in treatment which have been introduced since that series was studied. Particularly to the introduction by FAIRLEY (1930, 1932) of high protein low carbohydrate low fat diets and to the exhibition of B vitamin supplements.

The transitory nature of treated tropical sprue fits in with what might be expected of an infective process. It tends to support the hypotheses of STANNUS (1942) and LEISHMAN (1945) that the condition may be caused by an infection upsetting the intestinal bacterial flora and leading to impaired bio-synthesis of B vitamins, which in turn may catalyse various absorptive processes.

SUMMARY

1 In a series of 47 cases of tropical sprue it is noted that there was a tendency for the cases to occur in small groups among personnel of individual units.

2 The months of greatest incidence of sprue in this series were those in which amoebic dysentery in the district was most prevalent—a time when intestinal infections are especially common. This and the previous point suggest that tropical sprue may be an infective disorder.

3. The possible infective agents would appear to be other than the organisms producing amoebic or bacillary dysentery

4. After modern treatment the prognosis in tropical sprue over a 3-year period appears to be highly satisfactory. All 34 patients followed up were able to do their full duty—some heavy manual labour. The average increase in weight of the patients over the period was 24 lb. Any recurrences of sprue-like symptoms such as diarrhoea or ulceration of the tongue had been very infrequent and transitory.

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MALNUTRITION AND SNAKE POISONING IN THE SUDAN

BY

N L CORKILL,

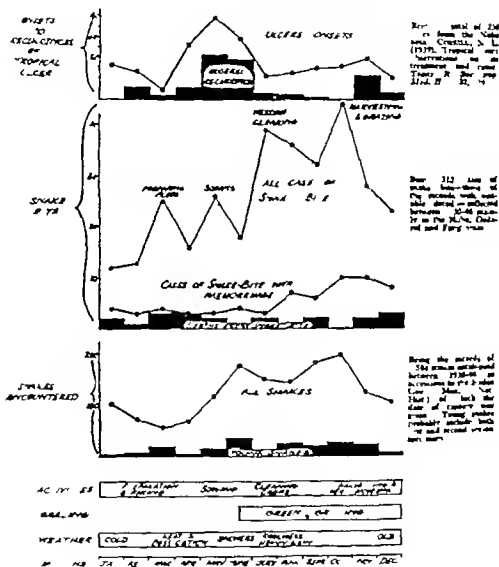
Liverpool School of Tropical Medicine, formerly Sudan Medical Service

In the Anglo-Egyptian Sudan the quite cold season (*shita*) is from mid-November to mid-February. The dry hot season (*sef*) is from February to May or early June. The cooler season of showers (*rashash*) is from late May fairly cold weather, extend from July to October. The October-November break before the cold winter is hot and humid.

Green grazing for stock and resulting from rain is available usually from mid-June in a decreasing extent to the end of December. It implies a good supply, with its important content of animal protein, carotenol, riboflavin, and, be it noted, ascorbin, also very greatly decreases in the hot dry season, especially in the absence of riverain or irrigated fodder-crops. (See graph.)

There are two main seasons of nutrient-deficiency affecting humans. One is the *rashash* (period of early rains or showers) when the peak of deprivation of milk and greenstuffs conducting to the development of pellagroid states, hyporiboflavinosis and scurvy, has superimposed upon it the cooling effect of the showers, acting as a stimulus to metabolism, and thus serving to excite overt manifestations of these states. The second and minor season of deficiency manifestations is in the winter when the severe cold acts as an exciting factor, causing latent deficiency states to become overt. This is the season when

ANGLO-EGYPTIAN SUDAN 1898 TO 1948: SOME INTER-RELATIONSHIPS OF SEASON
OCCUPY TO SEAF POUNDING AND MAGNITUDE



canthal glazing, cheilosis, follicular hyperkeratosis and mosaic skin are pronounced, especially in children.

The four bursts of agricultural activity are (a) firing and clearing of plots in the dry season, (b) sowing in the period of showers, (c) two weedings and clearings fairly close together in the early part of the heavy rains, and (d) harvesting after the rains. The month or so marking the closure of the rains and immediately following it is characterized by movement with herds to seek out all diminishing grazing, and living and sleeping out with as yet unharvested crops, to protect them from depredations, animal and human.

It is seen that snakes characteristically aestivate, and that most are encountered at the height of the rains, further, most young snakes are encountered in the early rains.

Cases of snake-bite, as might be expected, occur mostly during the rains when most snakes are abroad, and the four peaks clearly suggest that the four bursts of agricultural activity increase the danger of encountering snakes in their haunts. The tendency shown in the graph (the series is statistically small) for deaths from snake bite to be more in the dry season although the number of cases is less, and to be fewer at the height of the rainy season, although the wet season when milk and vegetables are plentiful, and the superimposed snake poisoning—apart from the synergistic haemorrhagic effect of viperine venom with ascorbin deficiency—serves through trauma as a pressor episode, *i.e.*, as a stimulant to katabolism, and thus tends to activate nutrient deficiency conditions, thereby increasing the illness of the bitten person.

There is much folk-lore to support this suggestion, it is sufficient perhaps to mention (a) the esteem in which milk, lemons, onions and fennel are held as snake-bite remedies in the Sudan—fennel has a particularly high value in ascorbin—and (b) the beliefs commonly held in the Sudan that vipers are dangerous in the hot season but not in the "rains" and that some (better fed?) communities are relatively little affected by their poison, whilst others (the worse fed?) are affected fatally.

Another common belief in the Sudan—and elsewhere in hot countries—is that snake-bite symptoms (and other bites and injuries) recur on anniversaries of the bite. The explanation would seem to be that persons suffering from ascorbin deficiency, most likely in the vernal season (spring in Europe and the *rashash* in the Sudan), who sustain injuries which tend to break down in the place at all, have inefficiently healed tissues which tend to break down in the next season of deficiency—to the simple mind the anniversary—or on the advent of a pressor episode (PETERSEN, 1938), if the ascorbin deficiency is chronic. The uppermost item in the composite graph above shows that tropical (indolent) ulcers in the Nuba Mountains of the Sudan have their maximal incidence and recurrence in the Sudan vernal season, the *rashash*.

ANSON's account of old wounds breaking out afresh in his scurvy ridden crew, LIVINGSTONE's story of recurrence of symptoms in old lion-bites, King Arthur's wounds, inflicted by Mordred and breaking out afresh yearly and the occurrence and recurrence of the bleeding stigmata of ultra-pious Christians, particularly in the spring after fasting and those of Moslems and Hindus, may all be considered as having their origin in this phenomenon. A Sudanese poet has written in the Song of Hardulla Abu Sen of the Shukria —

" The wounds (which you give me) like the wandering ostriches
visiting the rain-pools,
Though gone in the winter (*shibe*) return in the early rains
(*reshash*).

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SCLEROMA (RHINOSCLEROMA)

BY

LUDWIG JAFFÉ, M D,

United Fruit Company Hospital, Almirante, Republic of Panama

The following case of a 24-year-old Mestizo labourer from Nicaragua, was treated at intervals from 5th July until 18th October, 1946, in the hospital and out-patient department of the United Fruit Company in Almirante, Panama

HISTORY Impaired nasal respiration for several years Cough of recent origin

GENERAL PHYSICAL EXAMINATION No findings of significance

OTORHINOLARYNGOLOGICAL EXAMINATION *External nose*, normal *Anterior rhinoscopy*, both nostrils almost closed by scar-like tissue, much crusting *Posterior rhinoscopy*, the inferior part of the choanae closed by similar tissue *Oropharynx*, normal findings *Larynx*, the free edge of the right vocal cord appears to be irregular, probably due to granulations, both vocal cords are slightly reddened and thickened but move freely, there is mucous secretion and possibly some thickening of the mucous membrane of the subglottic area, voice hoarse *Ears*, left ear normal, right ear, drum membrane much retracted, thickened, handle of malleus slightly injected

LABORATORY FINDINGS Kahn negative Radiograph of the chest and the paranasal sinuses unremarkable Other laboratory findings like thick smears of the blood for malaria, nasal smears for *Leishmania* and *Mycobacterium leprae* and the sedimentation rate were normal Urine normal There was a slight hypochromic anaemia

Scleroma was suspected and sufficient tissue was removed from both nostrils to assure an adequate airway and different parts of the material sent to two pathologists One of them reported "Granuloma of undetermined aetiology Several areas of acute necrosis" The other pathologist, Professor RUDOLF JAFFÉ, Carácas, Venezuela, gave the following report "Granulation tissue infiltrated by round cells and many plasma cells In the granulation tissue many light transparent cells with foamy protoplasm Diagnosis Typical rhinoscleroma"

TREATMENT: During the first week sulphathiazol granules 33 were given. This was followed by several courses of foundain, or its Winthrop brand, repodal. Altogether the patient received during the 13 months of observation 180-50 c.c. of the drug by intramuscular injection. During that time he was also given 43-50 c.c. of 2 per cent. solution of tartar emetic by the intravenous route. Apart from these treatments and the surgical procedure in the nose therapy was entirely symptomatic and consisted mostly in roborts. Foundain or repodal were well tolerated but tartar emetic less so. The nose improved very much in the beginning of the observation but this possibly was due to the surgical removal of obstructing scleromatous tissue. An improvement of the laryngeal process was never obvious. There were also recurrent attacks of acute oedema which yielded to local heat application. Towards the end of the observation the nose became worse again and the affection of the larynx caused dyspnoea and stridor. The patient was advised to seek Roentgen, and, or radium treatment elsewhere.

BELINOW (1936) considers Central America and Sumatra the only "autochthonous" foci of scleroma besides those in the Pripiat Valley (Volhynia and Galicia) in South Eastern Europe. However old endemic foci in the Far East do not seem to be restricted to Sumatra. NOOSTEN KUNSCHEVER and Vos (1934) found the disease in Bali. OOSTEN and KUNSCHEVER (1938), another focus in Celebes. HAULUSKY and SARDJITO (1939) encountered the condition in Flores. According to SNIPPER (1936), scleroma is also found in nearer British India in Chota Nagpur. Whereas the disease is usually encountered amongst people of a low economic and hygienic standard, KUTLMAN KAISER and SARDJITO (1937) found it in the Pasoernah Lands in Sumatra among Mohammedans of a relatively high standard of living. According to STRUTT (1928), the first cases from Central America were reported in 1894. Conditions in Central America do not permit a full estimate of the extent of scleroma in these regions. It is certainly not uncommon in Guatemala and El Salvador. In Honduras the writer saw two cases (one of them already mentioned in a former publication (1942)), which showed the typical signs of scleroma. Several others were suspicious. Due to unfavourable external circumstances in none of these cases, histological or bacteriological verification was possible. Possibly some cases are not diagnosed because they do not resemble the impressive pictures of the books.

Scleroma is a chronic specific granuloma of the respiratory tract. Since the nose is not always affected the term scleroma is now preferred to rhinoscleroma. The disease is characterized by hypertrophic and atrophic processes of the mucous membranes. The nose, the pharynx and the larynx altogether may be found affected as well as each organ alone or a combination of two of them. Scleroma may also spread to the trachea and the bronchial tubes. Sometimes thickening of the external nose may be remarkable. In rare cases the skin of the nose, lips and cheeks may become diseased also. Obstruction of the nose and the nasopharynx may trouble the patient considerably and impede also the ventilation of the middle ear through the Eustachian tube. Affection of the larynx, trachea and bronchi may endanger life. The infectivity is low. Whether or not there is a relation to ozaena and atrophic rhinitis has not been established yet. The von Frisch bacillus (*Klebsiella rhinoscleromatis*) is recognized as the causative agent by some authors like BELINOW (1936).

LUDWIG JAFFE

Other writers like CUNNING and GUERRY (1942) consider the role of the bacillus unproved. In most cases the diagnosis can be verified by biopsy which shows the characteristic foam cells of Miculicz and hyaline bodies of Russel, but one should realize that there are periods in which the characteristic elements cannot be found yet or have already disappeared. According to BELINOW (1936), the complement-fixation reaction seems to be reliable and constant. Without carefully performed rhinoscopy and laryngoscopy, followed by biopsy, the disease may remain undiagnosed. As in so many less common entities it is important to remember the possibility of such occurrence.

There is no entirely satisfactory therapy. For treatment surgery, Roentgen, and/or radium, irradiation, tartar emetic and gold therapy have been recommended. LEIMENA and SARDJITO (1936) found in two cases foudadin more effective than tartar emetic. In our case a therapeutic effect of foudadin was not obvious. However, it was much better tolerated than tartar emetic. CUNNING and GUERRY (1942) tried also sulphonamides, which seemed to control the secondary infection but had no effect on the scleromatous tissue.

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A CASE OF THROMBOANGIITIS OBLITERANS IN AN AFRICAN WOMAN

BY

MARK HUGHES, B M , B CH ,
Medical Officer, Colonial Medical Service, Gold Coast

Thromboangitis obliterans is uncommonly seen in women, and has very rarely been reported in Africans, or in American negroes of African descent. The case described below is believed to be the first published record of the disease in a negress

Case Report

Azinana, aged about 35 years, was a woman of the Frafra, or Nankanni, tribe, who had never left her home district in the Northern Territories of the Gold Coast. On 12th September, 1947, she was admitted to Navrongo hospital, in the Northern Gold Coast, with a chronic ulcer of the left foot, stated to have been present for over 2 years. The onset of the condition had been gradual, and had been preceded by pain and swelling after walking long distances, but no history of intermittent claudication could be elicited. The patient habitually chewed, and smoked, a considerable amount of tobacco, as is usual with both sexes of her tribe, she stated that a ball of tobacco about the size of a grapefruit, the usual unit of trade in this commodity, lasted her from 7 to 10 days. No accurate history of the course of her illness, or of previous disease, could be given by the patient.

The distal part of the patient's left foot was absent from the mid-metatarsal level. The blackened heads, and half the shafts, of the sequestering metatarsal bones projected from a fairly clean granulating surface. The proximal part of the foot was somewhat swollen, and there was an ulcer 1 inch (2.54 cm.) in diameter on the plantar aspect of the heel. The anterior tibial artery could not be felt on the left ankle, but on the right ankle pulsations were within normal limits. No thickening of the radial arteries was detected.

General physical examination of the patient revealed nothing abnormal except a severe degree of anaemia. The haemoglobin was 30 per cent by Tallqvist's method (no haemoglobinometer was available). There was no sugar or albumen in the urine. The blood film showed no parasites. There were no helminth ova in the stool.

Antiseptic dressings were applied to the foot, ferrous sulphate and ascorbic acid were given by mouth, and injections of liver extract were begun. After 3 weeks the haemoglobin was still only 35 per cent, but the ulcers were clean, and the granulations bled when touched. It was thought that healing might take place, and the sequestering metatarsals were therefore removed under general anaesthesia. This interference was followed by a regression in the local condition, 2 weeks later the ulcer on the heel had increased in size, in a further week signs of gangrene appeared in the skin of the lower part of the left leg.

Seven weeks after admission the haemoglobin was still only 35 per cent, in spite of continuous iron and liver therapy. The patient's appetite had deteriorated, and her general condition was giving rise to anxiety. On 18th November, 1947, the left lower limb was amputated through the thigh, 10 inches (25.4 cm.) below the anterior superior iliac spine. Healing by first intention took place, accompanied by a dramatic improvement in the patient's general health. The appetite returned, and the haemoglobin began to rise. By the 6th January, 1948, the haemoglobin had reached 70 per cent. When the patient first attempted to walk with crutches, oedema and pain occurred in the right foot, these symptoms disappeared later, but may have been indications of circulatory insufficiency in the right foot.

* Thanks are due to Dr G. ROBINSON for the pathological report, and to the Director of Medical Services, Gold Coast, for permission to publish this case.

The femoral artery in the amputated leg appeared normal. The anterior tibial vessel was thrombosed at its origin from the popliteal artery. A specimen, including the bifurcation of the popliteal artery was forwarded to the Medical Research Institute at Accra for section.

Pathological Report

Dr G. ROBINSON, Senior Pathologist, Gold Coast Colony kindly sectioned and examined the specimen. He reported upon it as follows:

"This appears to be a case of thromboangitis obliterans (Buerger disease). One vessel is closed by fibrous tissue which is partly canalized.

DISCUSSION.

The diagnosis of thromboangitis obliterans was substantiated by the histological findings in the anterior tibial artery. It was unfortunate that the Kahn test was not done on this patient, although the presence of endemic yaws in the district would have detracted from the significance of a positive result. The association with tobacco was noteworthy. The severe anaemia was probably the result of toxic absorption from anoxic and gangrenous tissue, since it did not respond to iron and liver therapy until after amputation.

Ten cases of thromboangitis obliterans in full-blooded negroes have been reported in the literature by GRIMMILL (1925), SMITH (1936), SCURHAM (1936), YATER (1937), WARIKAWEXY (1941), and by GOETZ in a personal communication quoted by GELFAND (1947). Only the last case (Goetz), of which no details were published occurred in Africa, the remainder being in American negroes. In addition, PARSONS (1936), and MORTON (1938) each recorded a case in a half-caste. All these cases were males, and in some the diagnosis was not histologically confirmed.

Four of YATER's cases were seen at the same hospital within 27 months, supporting his suggestion that the incidence of this disease in negroes will be found to be not so rare as it seems. The dearth of published records of angitis obliterans from Africa is but a poor indication of the rarity of the disease for doctors are lamentably scarce in that Continent. The early onset of the condition could easily escape notice in a country where ulcers are so common and so frequently treated by semi-trained auxiliaries. angitis obliterans is extremely uncommon in women of all races. saw only three women in a series of 500 cases of the disease. (1948) collected 31 cases in females from the literature, two records of their own. None of these 33 patients was

CORRESPONDENCE.

To the Editor, TRANSACTIONS of the Royal Society of Tropical Medicine and Hygiene

THE EFFICACY OF PALUDRINE (PROGUANIL) AS A THERAPEUTIC AGENT

(WEST AFRICAN AND NEW GUINEA STRAINS OF *Plasmodium falciparum*)

SIR,

A paper by COVELL, NICOL, SHUTE and MARYON (1949) was published in the March number of these TRANSACTIONS (42, 465) concerning the efficacy of paludrine as a therapeutic agent in patients infected at the Horton Hospital, Epsom, with a West African strain of *P. falciparum*. Five patients with overt primary *falciparum* malaria received paludrine mg 300 once daily for 14 days, and five other patients received mg 300 twice daily for 7 days, of these ten patients, nine had recrudescences within 3 weeks. When this treatment, *i.e.*, paludrine gramme 0.6 daily for 10 days was reinforced with either quinine (gramme 2.0) or mepacrine (gramme 0.9) on the first day of treatment, radical cure resulted.

The failure to produce radical cure in M.T. malaria with a single course of paludrine was in marked contrast to results obtained at the L.H.Q. Australian Medical Research Unit, Cairns. In a footnote to their paper (page 472), COVELL and his colleagues suggest that the high rate of radical cure obtained with paludrine by FAIRLEY *et al* (1946)* in *falciparum* infections from New Guinea may have been due to delayed treatment, and that if this were so the two series would not be strictly comparable.

In the Cairns series, treatment with paludrine was instituted at variable

* *Trans R Soc trop Med Hyg*, 40, 105

periods after exposure to infection, from the time sporozoites first entered blood on the seventh day to several weeks after the onset of overt malaria.

That delayed treatment and the development of partial immunity not the factor responsible for radical cure was clearly demonstrated in our earliest experiments with the New Guinea strain of *P. falciparum* (p. 129 to 131). In these experiments six volunteers were exposed to 19 infective bites with a New Guinea strain of *P. falciparum*. On the seventh after exposure subinoculations were made from each of the six volunteers, 20 of blood being directly transfused into a non-immune recipient—all the recipients subsequently developed overt falciparum malaria, showing that the parasites had been present in the blood of the original donors on the seventh. Immediately after the subinoculations on the seventh day treatment commenced—three of these volunteers (Group CVIIA) received paludrine mg. daily for 14 consecutive days, and the three other volunteers (Group CV) received mg. 300 daily for the same period. Parasites were never found in blood smears of any of these sporozoite-inoculated volunteers and none developed fever or other clinical evidence of malaria. The subsequent course of events revealed that all six had been radically cured by paludrine mg. 100 or 300 to daily for 14 days. In these volunteers treatment was instituted at the earliest possible moment after merozoites had entered the blood stream. It would be impossible in terms of present knowledge to devise an experiment more effectively designed to eliminate the possibility of immunity playing any role in the therapeutic result.

In the Cairns series radical cure was obtained in 45 out of 48 experimental sporozoite infections with *P. falciparum* and in 41 out of 41 natural infections with *P. falciparum* contracted in New Guinea. The average duration of asexual parasites demonstrable in thick films was 2.35 days from commencement of treatment in the experimentally infected group, and 2.1 days in the naturally infected group. As COVELL and his colleagues point out, details regarding the time of commencing treatment in the overt attack were not stated in our paper. In 16 out of these 48 experimentally-infected volunteers, paludrine administration started from 1 to 5 days after the onset of fever—the mean being 2.7 days. Fifteen of these 16 infections were radically cured. The single failure was a volunteer to whom paludrine mg. 100 was first administered 6 hours before exposure to infection. He developed demonstrable parasites in the blood 12 days later and fever the next day. Five days after primary fever had commenced, a course of paludrine consisting of mg. 100 thrice daily for 10 days was inaugurated. Parasites were found again microscopically on the 45th day—subsequently a course consisting of paludrine gramma 1.0 daily for 14 days produced radical cure.

Actually those volunteers who were left untreated in the primary falciparum attack until parasite densities in the peripheral blood approximated 300,000 per c.mm. were not easier—but more difficult to treat than their fellow

who were treated earlier when the parasite densities were still around 100 to 1,000 per c mm. Over this 3 to 5 day period evidence of immunity was never detected.

Though specific data, obtained from the later paludrine-treated cases in the series under review, did not indicate that immunity from delayed treatment was a significant factor in their radical cure, the claim for the extraordinary efficiency of paludrine in radically curing infections with the New Guinea strain of *P. falciparum* can be equally well based on the results obtained in early treated patients which are comparable to the Horton series. Unpublished observations by BLACKBURN show that immunity did develop in certain other *falciparum* infections at Cairns, but when it did, the period of time required was measured in terms of weeks, not in terms of days.

Six out of six volunteers treated in the earliest stages of parasitaemia when merozoites had just entered the circulating blood, and 15 out of 16 patients treated in the early stage of primary malaria fever were radically cured by a single course of paludrine, these findings exclude the possibility that immunity played any role in the radical cure of these infections with the New Guinea strains of *P. falciparum*.

All these volunteers at Cairns were of the Caucasian stock. None of them had served in New Guinea or other areas in the Pacific, and none had suffered from overt malaria previously. The extreme sensitivity of the New Guinea strains of *P. falciparum* to the schizonticidal action of paludrine was indicated by —

(1) The remarkable response often elicited by one single small dose (mg. 100) in non-immune volunteers leading to clinical cure and temporary disappearance of asexual parasites.

(2) The very high incidence of radical cure obtained in natural as well as experimental infections following paludrine 0.1 thrice daily for 10 days.

(3) The rapid clearance of asexual erythrocytic parasites during such a course of treatment (2.1 and 2.35 days).

The most reasonable explanation of the different therapeutic response is that we are dealing with two different geographical strains of *P. falciparum*, the New Guinea strain being very sensitive and the West African strain relatively insensitive to the schizonticidal action of paludrine. Difference also exists in regard to the schizonticidal action of quinine, since grain 5 appeared to produce complete suppression in infections with the West African strain, whereas grain 10 invariably failed to suppress experimental infections with the New Guinea strain of *P. falciparum*. The West African strain is obviously more sensitive to quinine than the New Guinea strain—a fact which was all too evident in the New Guinea campaigns of 1942.

During the recent war two other strains were found in New Guinea differing significantly from most other strains previously described —

(1) The New Guinea strain of *P. vivax*, which has a very close relapse pattern, the interval between primary fever and the first relapse varying from 3 weeks to 3 months in most instances, even quinine sulphate grain 10 often failed to suppress benign

tertian fever and both primary attack and relapses were very difficult to cure except with plasmoquine in combination with quinine or paludrine. This strain is probably identical with the "Chesson strain" which, in the United States of America, is thought to have originated in New Guinea.

(2) The Iupe-Wewak strain of *P. falciparum*, which was relatively insensitive to mepracrine. This strain required up to grammes 0.2 daily to suppress fever and radical cure was not necessarily produced even with this dosage. It was also more resistant to therapy during overt attacks, larger dosage of mepracrine being required to produce radical or even sometimes clinical cure. It readily responded to paludrine therapy. This strain was also not suppressed by quinine grain 10 daily but it was normally sensitive to both paludrine (mg. 25 daily) and resorcin (mg. 80 daily). Had either of these drugs been available no difficulty in controlling the Iupe Wewak outbreak in New Guinea in 1945 would have been encountered.

When discussing the New Guinea strain of *P. vivax* described by FAIRLEY and his colleagues (1945) JAMES (p. 458) said "Indeed, his results in general support the view expressed at a meeting of our Society about 10 years ago that, from the point of view of drug prophylaxis and treatment, strains may be more important than species."

In none of the three New Guinea strains was there an opportunity of demonstrating that they were immunologically distinct from Indian, African, or European strains of *P. falciparum* or *P. vivax* as the latter were not available at Cairns. New Guinea, however, is situated well within Wallace's line, and under such circumstances it is perhaps not surprising that certain differences exist in the geographical strains of *Plasmodium* found there.

I am, etc.,

N. HAMILTON FAIRLEY

London

10th May 1949

Trans R. Soc. trop. Med. Hyg. 43, 311

ELECTRICAL CHARGE OF TRYPANOSOMES

SIR,

An article entitled "A New Approach to Trypanosomiasis," by FAIRBURN and CULWICK, *Annals of Tropical Medicine and Parasitology* (1946), is provocative from its title to its final paragraph, yet it appears to have been accepted without comment. Of the many interesting contentions, I wish to deal with only one—that the distribution of trypanosomes in a very thin blood film is related to their electrical charge—positively charged parasites being in contact with erythrocytes, while negatively charged ones are repelled from and do not touch the red cells.

In view of the very short time required for the making of a thin film, and the relatively terrific mechanical forces to which the blood ingredients are submitted when the blood is spread, it would appear strange that trypanosomes

—which are not adherent to red cells in the circulating blood—should within a second or two of release from the circulation, and while submitted to violent mechanical disturbance, be able to orientate themselves in any way that is not dictated by this disturbance. However, it was with an entirely open mind that I tried to confirm FAIRBAIRN and CULWICK's contention, and as I have failed to do this I write to ask other observers what they have found. Silence may be interpreted as consent, and since the article aroused no open dissension it was natural that FAIRBAIRN (1947), in a more recent article, should write about trypanosomes being classified "as positively or negatively charged, as described by FAIRBAIRN and CULWICK (1946)," as though he were remarking on an approved procedure.

My observations were made with the Tinde strain of *Trypanosome rhodesense* maintained at Mpwapwa in guinea-pigs. Although no cyclical transmission had taken place for more than a year, the parasites showed fully normal variations in morphology and electrical charge. Consequently, it was easy to select animals in which a great predominance of their numerous trypanosomes carried a charge of one sign, when this was determined by the method of BROOM, BROWN and HOARE (1936), a method that gives objectively clear-cut results. For example —

On 28 8 48,	97 per cent	of the trypanosomes of G P No 1	were negatively charged
26 8 48,	90		1
4 9 48,	93		3
6 9 48,	97		3
7 9 48,	86		1
9 9 48,	88		3
9 9 48,	96		2
17 9 48,	100		2
17 9 48,	100		4

and so on until the last observations, made on 18 3 49, showed 100 per cent of the trypanosomes of both G P No 24 and G P No 25 to be positively charged.

With such ample material it was very easy to obtain thin smears from animals of which the trypanosomes were predominantly negatively or positively charged, and to compare the smears. It is certainly significant that whereas there was no disagreement between observers regarding the nature of the charge on trypanosomes suspended in glucose-saline, there was no agreement at all between observers who used the thin film method advocated by FAIRBAIRN and CULWICK. To take one, but not extreme, case. Two observers agreed that approximately 97 per cent of the trypanosomes of G P No 1 were negatively charged in glucose-saline, but the same two men returned 93 and 29 per cent respectively as the percentage of negatively charged trypanosomes as judged by positions on films.

In the end none of us here could escape the conclusion that the positions of trypanosomes in thin films are governed entirely by the action of the glass spreader. The closer together the elements of the blood, the more likely are

the trypanosomes to touch cells the more widely the red cells are scattered, the more likely is a trypanosome to be detached from all other cellular elements.

I am, etc.,

H. E. HOSNEY

Veterinary Research Laboratory
Mtwapa, Tanganyika Territory
23rd March, 1949

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LYMPHOSTATIC VERRUCOSIS

SIR,

The letter by Dr WHITE (1949), in your last issue, and Dr CLARK's (1948), referring to the above condition, prompt me to offer some comment.

The photographs contributed by Dr WHITE are very suggestive of chromoblastomycosis, *i.e.* the original "mossy foot" of WOLFFSTAN THOMAS. When I wrote my paper on lymphostatic verrucosis in 1934 I had not seen cases of chromoblastomycosis, but I was certain that the condition I described had no relation to this disease. Since coming to South Africa I have seen several cases of chromoblastomycosis, and suggest that Dr WHITE's case is readily recognizable.

Regarding Dr CLARK's speculation concerning the aetiology of true lymphostatic verrucosis, it has occurred to me that a condition such as tropical primary phlebitis (FISHER, 1941; GELFAND 1949) might be the original cause of the oedema. I agree with Dr CLARK's conclusions regarding the absence of any other obvious cause of oedema and should like to congratulate him on his contribution.

I am, etc.

L. J. A. LOEWENTHAL

209 Jeppe Street,
Johannesburg
15th March 1949

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LYMPHOSTATIC VERRUCOSIS

SIR,

I was very interested to read Dr WHITE's letter in your TRANSACTIONS of January, 1949 (No 4, Vol 42), in which he suggests that lymphostatic verrucosis might be caused by *S haematobium*. It is, of course, more than possible that this interesting condition is a secondary manifestation of some other disease, and it is only because I have been unable to connect it with any other condition that I have come to regard it as a clinical entity.

S haematobium cannot, however, be the cause—in this district at any rate—as it does not occur here. *S mansoni* occurs only in one corner of the district far from the homes of many of my patients.

It is, however, possible that Dr WHITE's case is not the same condition as we find so commonly near Mount Kenya and the Aberdare Mountain Range.

I see from his photographs that the foot strongly resembles the condition which is so common here and is usually known as lymphostatic verrucosis. But the verrucose condition extends up to the knee while in no case that I have seen here does the verrucose condition stretch for more than an inch or two above the ankle.

A close study of Dr WHITE's photographs also makes me think that the verrucose condition is rather different from the one I am so familiar with—it appears to be more patchy and discrete, and more raised from the surface of the skin than occurs in the cases I see.

The condition is a most interesting one and one we appear to know little about.

I am, etc ,

Fort Hall,
Kenya

MALCOLM CLARK

11th March, 1949

INDEX TO AUTHORS

- Adams, A R D, and Seaton, D R, 314
 Ainsworth, G C, 223
 Alakija, O B, 338
 Alves, W, 115
 Andrews, W H H, with Maegraith, B G, Tottey, M M, Townshend, R H, and Wenyon, C E M, 312
 Apted, I, Harding, R D, and Gosden, M, 55
 Armstrong, T G, Wilmot, A J, and Elsdon-Dew, R, 597
 Ashbel, Rivka, 409
 Ayyad, Naguib, with Azim, M A, 231
 Azim, M A, and Ayyad, Naguib, 231
 ———, with Watson, J M, and Halawani, A, 37
 Bahr, P Manson- See Manson-Bahr, P
 Barnes, M E, 199
 Baylis, H A, 531
 ———, Manson-Bahr, P, and Buckley, J J C, 323
 Bennet, J, 437
 Bernkopf, H, Stuczynski, L A, Gotlieb, T, and Halevy, Ch, 259
 Bertram, D S, 318
 ———, and Roberts, E W, 8
 ———, Kershaw W E, and William-son J, 319
 Birks P H, 203
 Black, R H, 9, 565
 ———, with Tottey, M M, 313
 Blackburn, C R Bickerton, 117
 Blair, Dyson M, 322
 ———, with Goodliffe, F A, 205
 Brosius, Barbara, with Brosius, O T, and Thomas, E E, 95
 Brosius, O T, Thomas, E E, and Brosius, Barbara, 95
 Brotherston, J G, 10
 Buckland, F, 3
 Buckley, J J C, with Baylis, H A, and Manson-Bahr, P, 323
 Busvine, J R, 6
 Cameron, T W, 415
 Cannon, D A, 322
 Cawston, F G, 507
 Chesterman, C C, 115, 427
 Chwatt, L J, with Gordon, R M, and Jones, C M, 315
 Clark, Malcolm, 287, 629
 Cockburn, T A, 291, 509
 Cooke, W E with Manson-Bahr, P (title only), 323
 Corkill, N L, 613
 Covell, G, Nicol, W D, Shute, P G, and Maryon, M, 341, 465
 Crewe, W, with Gordon, R M, 7, 316
 ———, and Maegraith, B G, 317
 Crowden, G C, 325, 340
 Crusz, H, 322
 Dawson, J, Findlay, G M, and Ward, R D, 277
 Day, B, 114
 Deeb, A A, with Erfan, M, Erfan, H, and Mousa, A M, 477
 Derrick, E H, 191
 Dew, R, Elsdon- See Elsdon-Dew, R
 Dukes, C E, and Morgan, C N, 14
 Duncan, J T, 207, 225
 Earle, K Vigors, 101
 Early, E P M N, 438
 Edeson, J F B, with Field, J S, 569
 Elsdon-Dew, R, with Armstrong, T G, and Wilmot, A J, 597
 Ercole, Q N, and Mackerras, M J, 443, 455
 Erfan, M, 109
 ———, Erfan, H, Mousa, A M, and Deeb, A A, 477
 Erfan, H, with Erfan, M, Mousa, A M, and Deeb, A A, 477
 Evans Electro selenium, Ltd, 3
 Fairley, N Hamilton, 623
 Field, J W, and Edeson, J F B, 569
 Findlay, G M, 13, 525
 ———, with Dawson, J, and Ward, R D, 277
 Fourman, L P R, 299
 Fulton, J D, Joyner, L P, and Niven, Janet, 9
 Garnham, P C C, 321, 527
 ———, with Shortt, H E, 7, 321
 Gebert, S, 295
 Gelfand, M, 283
 ———, and Ross, W F, 559
 Gibbs, Alfred J, 89
 Girdwood, R H, 65
 Goodliffe, F A, and Blair, D M, 205
 Gordon, R M, Chwatt, L J, and Jones, C M, 315
 ———, and Crewe, W, 7, 316
 Gosden, Minnie, with Apted, I, and Harding, R D, 55

- Gotlieb, T., with Bernkopf, H. Stue-
 zynski, L. A. and Halvry Ch.,
 259
 Goyal, R. H., 631
 Grundy J. H. 3

 Hackert, C. J. 329
 Haleward, A., with Watson, J. M., and
 Axim, M. A., 67
 Halvry Ch., with Bernkopf, H., Stue-
 zynski, L. A. and Gotlieb T.
 259
 Hare, K. P. 439
 Harding, R. D. 347
 ——— with Apted, I. and Gooden
 M., 85
 Harrison, J. L., and Woodrille H. C.,
 347
 Hawking, F. Perry W. L. M., and
 Thurston, J. P. 16
 ——— with Perry W. L. M., and
 Sewell P. E. J. 10
 Himsworth, H. P. 422.
 Hindle E., 31
 H. Eutrope A. Soong, Tsung-Hsun,
 and Li, Young, 573
 Hoare C. 32.
 Hornby H. E., 626
 Horton-Smith, C. 11
 Hughes, M. H. 631

 Jaffé, Ludwig, 617
 Jones, C. M. with Gordon, R. M., and
 Chwatt, L. J. 318
 Joyner L. P. with Fulton, J. D. and
 Niven, J. 6

 Kent, R. P. with Murgatroyd, P. 16
 Kershaw W. E., 318
 ——— with Bertram, D. S., and
 Williamson, J. 316
 King, E. J. 4
 Kirk, Robert, 501
 Kosmidt, J. A., with Payne, E. H. and
 Sharp, E. A., 183
 Koenigslein, R. P. 503

 Lamborn, W. A., 320
 Lewis, D. J. 393.
 Li, Young with Ho, Eutrope, A., and
 Soong, Tsung Hsun, 573.
 Loewenthal, L. J. A., 626.
 Laurie E. M. (Chalmers Medal
 Award) 107
 ——— and Scatton, D. R., 315
 Low O. Carmichael, 34

 McCarthy D. D. and Wilson, D.
 Bagster 83
 McKenzie A., 426.

 McRobert, George, 324 337 426
 Mackerras, M. J. and Ercole Q. \,
 443, 465
 Macraith, B. G. 327
 ——— Andrews, W. H. H. Tetter
 M. M., Townshend, R. H. and
 Weyron C. E. M., 612.
 ———, with Crew, W. 317
 Mahaffy A. P. 611 529
 Mannfold, J. H., 3
 Marston-Bahr P. and Cooke, W. E.
 (title only), 323
 ——— with Baylis, H. A., and Buckley
 J. J. C. 323
 Maryon, M. with Covell, G. Nicol,
 W. D. and Shute P. G. 641 463
 Mattingly P. F. 529
 Maurice, H. G. 26
 Meneses A. N. T. with Sachs, A.
 Beyers, M. H. P. and O'Dwyer
 J. J. 1
 Moore D. Fitzgerald, 416
 Morgan, C. N. 14
 Morgan, M. T. 336
 Morton, T. C., 25
 Moua, A. M., with Erfan, M., Erfan,
 H., and Deeb A. A., 477
 Mucode, I. 318, 323.
 Murgatroyd, F. and Kent, R. P. 16

 Nagay H. P. and Zanety A. P. 493
 Napier L. E., 423
 Nicol, W. D. with Covell, G. Shute
 P. G. and Maryon, M., 341 463
 Niven, Janet, with Fulton, J. D. and
 Joyner L. P. 6

 O'Dwyer J. J. with Sachs A.,
 Meneses, A. N. T. and Beyers,
 M. H. P. 1

 Pal, Rajindar 6.
 Passmore, R., 347
 Payne E. H., Sharp, E. A., and
 Kosmidt, J. A., 183
 Perry W. L. M., Sewell, P. F. J. and
 Hawking, F., 16
 ——— with Hawking, F. and Thurston,
 J. P., 16
 Pierce, A. E., 12.
 Priest, W. M., 331

 Rae, Wilson, 323.
 Ramsford, O. \ 267
 Rewell, R. E. 17 25 416
 Rice, H. M. 6
 Richards, W. S. 6
 Roberts E. W. 229
 ——— with Bertram, D. S., 6
 Rowe, W. F. with Gelfand, M. 336

Sachs, A, 2
 —, Meneces A N F, Sayers, M H P and O'Dwyer, J J 1
 Sayers, M H P, with Sachs, A, Meneces, A N F, and O'Dwyer, J J 1
 Schwetz, J, 403
 Scott, Harold, 27
 Scaton, D R, with Adams, A R D 314
 —, with Lourie, E M, 315
 Sewell, P F J, with Perry, W L M and Hawking, F, 10
 Sharp, E A, with Payne I H and Knaudt J A 163
 Shircort, J O, 508
 Shortt, H E, 227, 321
 —, and Garnham, P C C, 7, 321
 Shute, P G, 324
 —, with Coull, G, Nicol W D, and Maryon, M, 341, 465
 Smith, Dean A, 437
 Soong, Tsung-Hsin, with Ho, Eutrope A, and Li, Young 573
 Stannus, H S, 225
 Stuczynski, L A, with Bernkopf H, Gotlieb, T and Halevy Ch 259
 Laylor, E L, 12
 Tidy, H, 113, 239
 Thomas, E E, with Brosius, O I, and Brosius, B, 95
 Thomson, F Adam, 487
 Thurston, J, with Hawking, F, and Perry, W L M, 10
 Tottey, M M, and Black, R H, 313
 —, with Macgrath, B G, Andrews, W H H, Fownshend R H, and Wenvon, C E M, 312

Fownshend, R H, 314
 —, with Macgrath, B G, Andrews, W H H, Iottet, M M, and Wenvon, C E M 312
 Irowell, H C, 417, 439

Ungar, J, 33

Ward, R D, with Dawson, J, and Findlay, G M, 277
 Waterlow, J C, 434
 Watson, J M, Azim M A, and Halawani, A, 37
 Watts, J C, 339
 Wenvon, C M, 33
 —, Obiuary, 303, 309
 Wenvon C E M, with Macgrath, B G, Andrews, W H H, Iottet, M M, and Fownshend, R H, 312
 Weiner J S 336, 339
 White I H, 324, 410
 Willcox P H A, 171
 Williamson, J with Bertram D S, and Kershaw, W I, 319

Williamson, K B 13
 Wilmot, A J, with Armstrong, I G, and Fildon-Dew, R, 597
 Wilson, D Bigster, with McCarthy D D, 83
 Wilson, F, 205
 Woodman, H M, 543
 Woodruff, A W, 605
 Woodville, H C with Harrison, J, 247

Yochi, M, 99

Zanaty, A I, and Nagaty, H F, 493

INDEX TO SUBJECTS

Acclimatization, 336
 Acetylarsen, and yaws, 55
Aedes adersi, possible vectors of yellow fever, 527
 africanus, in yellow fever, 519
 deboeri, possible vectors of yellow fever, 527
 simpsoni, in yellow fever, 516
 Anaemia, in Indian troops, 65
 macrocytic, treatment by folic acid, 203
 and malnutrition, treatment, 376
Ancylostoma, in treatment of polycythaemia, 493

Anopheles gambiae, extermination in Wadi Halfa, 398
 maculipennis, oöcysts in, 324
 stephensi, oöcysts in, 324
 Antivenemics, standardization of, 381
 Amocbiasis, complicated by carcinoma, schistosomiasis and adenoma, 14
 liver abscess and chloroquine possibly due to *Iodamoeba butschli*, 191
 Ayerza's disease, 113

INDEX TO SUBJECTS

- Balantidium* in primates, 291
 Biological research, techniques for 312
Bismuth sodium potassium tartrate
 yew treated by 55
 Blood pressure: effect of climate 299
- Capillaria hepatica*, case report, 66
Cercopithecus aethiops infection by *Leish-*
mania 501
 Chalmers Medal, 1941 106
 1943 107
 1945 107
- Chloroquine liver abscess and, 16
Chrysops breeding places in British
 Cameroon, 618
 Clayton Lane, Lt.-Col., death of 410
Closterchus sinensis, epidemiology of
 503
 Colony counter 3
 Colonoscopy 6
 Correspondence—
 Bancroftian filariasis 306
 Electrical charge of trypanosomes
 626
 Fourth International Congress of
 Tropical Medicine and
 Malaria, 410
 Intestinal infections of primates,
 413 509
 Lymphomatous eruptions, 410 623
 629
 Macrocytic anaemias treated with
 folic acid, 603
 Mechanical purification of river
 water 507
 Paludine (proguanil) as thera-
 peutic agent, 623.
 Recurrent haemoglobinuria treated
 with sulphathiazole 508
 Schistosome miracidia, hatching
 speed, 505.
 Sporobolus pernici, 409
 Vitamin B₁₂ deficiency states, 413
Cystocercus puiformis, transverse
 flexion of in rabbits 323
- Damaeline (Bercakoe) boris* L. macra-
 cides, 6
 D.D.T. Detection of 6
 Anti anopheline protection,
 295
 Deficiency diseases, Vitamin B₁₂ defi-
 ciency Gold
 Coast, 277
 Marasmus, in In-
 dian troops,
 65
 Macrocytic anae-
 mia, treat-
 ment by folic
 acid, 503.
- Dengue relation to *Aedes* prevalence
 63.
 clinical features, 85
 Dietary deficiencies in F.J.I, 497
 teeth in, 498.
 Diseases, Tropical, in captive wild
 animals, 17
 Dysentery amoebic treatment of, 297
- Eimeria bovis*, drying of oocysts, 10
trachis, drying of oocysts, 10.
 sulphamezathine, 11
Entamoeba histolytica, hyaluronidase
 production, 314
 Extradural block in tropical ulcer 297
- Falciparum malaria, treatment of, 545
Fasciola hepatica miracidia in *Lymnaea*
truncatula, 320
 Filariasis bancroftian, periodicity in,
 308
 in cotton rat, 310
 chemoprophylaxis, 619
 in the Sudan, 643
- Folic acid, treatment of macrocytic
 anaemia, 503
 Fungus diseases, epidemiology of 307
 treatment of 315.
- Glaucis, sensitization to beta, 316.
- Haemophobinae, treated with sulphathiazole 508.
 Heat, hyperpyrexia, 2.
 Hyaluronidase production, *E. his-*
 tolytica, 614
H. dropobla, in U.K., 6
- Insectophaga armatipes*, in man,
 323 531.
 Insects, benzalyl and bites of 6
 blood-sucking, pool feeding
 618.
 Insecticides, louse of cattle, 6
 D.D.T. detection of 6
Iodamoeba butchlii, and amoebiasis,
 161
- Kala-azar disappearance of *Leishmania*
 under treatment, 573.
 experimental host, 6
 Kwashiorkor 417
 treatment of, 425
 liver in, 423
- Leishmaniasis*, in Sudan, 501
Leptomonas carmichaeli tolerance of
 humidity and salinity 86
Leptopora skrjabini megu-
 taxis, 261
Leptoparva, bovine, anti-bodies in,
 256

INDEX TO SUBJECTS

- Leishmaniasis* first stage 199, 318
 controlled infection in cotton rat, 319
- Loa loa*, infection rate, fly and human, British Cameroons, 315
- Lymnaea truncatula* culture of, 12
- Lymphostatic verrucosis*, 410, 628, 629 in Kenya, 287
- Macrocytic anaemia, in Indian sepoys, 367
- Malaria, in Central Africans, 403
 parasites in pituitary, 2
 parasites, non-pigmented in bone marrow, 99
 human parasites and culicine, 13
 trophozoites, origin of, 13
 See also *Plasmodium*
- Malnutrition, and snake poisoning, 613
 malignant (Kwashiorkor) treatment of, 417
 liver in, 433
- Marasmus in Indian troops, 65
- Meetings of the Society—
 1948
 18th March, Laboratory Meeting at R.A.M. College, Millbank, SW 1 1
 15th April, Ordinary Meeting, 17
 17th June, 41st Annual General Meeting, 105
 Ordinary Meeting, 109
 21st October, Ordinary Meeting, 207
 18th November Laboratory Meeting at the School of Tropical Medicine, Liverpool, 311
 9th December, Ordinary Meeting 325
- 1949
 20th January, Ordinary Meeting 417
 17th February, Ordinary Meeting 511
 Meningo-encephalitis in West African troops, 581
 Microfilariae identification of 547
 Miricid (Nalodim) anti-bilharzial treatment 37
 toxicity of 40
- Mosquitoes, *Aedes aegypti*, bites of, 7
 apparatus for studying, 6
 bites, generalized reaction, 317
 DDT sprays, 295
- Obituary C. M. Wenyon, 303, 309
- Ostrich, contents of gizzard, 29
- Palm leaf traps, for aquatic snails, 231
- Paragonimus*, eggs in sputum of patient in Cameroons, 322
- Parasites, malaria, respiration, 313
- Paludrine, prophylactic for *P. falciparum*, 341, 465
 as therapeutic, 623
- Photometer, 4
- Physiological studies of work in hot and humid environments, 325
- Plasmodium cynomolgi*, life cycle of, 227
 pre-erythrocytic cycle, 7
 tissue forms, 10
 schizonts, fourth day after infection, 321
- falciparum*, gametocytes
 anti-malaria drugs, 455
 paludrine forms, 9
 paludrine as prophylactic 341
 paludrine as therapeutic 465
- malaria, treatment of 473
 sp., in East African skin, 321
- rasalli*, exo-erythrocytic development of, 569
- trax*, anti-malarial drugs, 443
 chemotherapeutic activity of drugs, 151
 non-pigmented parasites, 99
 pre-erythrocytic stages, 7
trax tolerance and immunity 117
- Polythaemia vera treatment by *Draculostoma* infection, 493
- Preservation pathogenic organisms by freezing 10
- Primates intestinal infection, 413, 509
- Rats control of 247

- Ru er heater mechanical purification, 307
- Schistosoma haematobium* hatching speed of miracidia, 305
- Schistosomiasis*, diagnosed by rectal biopsy 223
incidence of, in South Central Africa, 359
in Southern Rhodesia, description of photographs, 222
pulmonary, 169 477
radiological study, 481
S. intercalatum 115
- Scleroma, 317
- Snake poisoning and malnutrition, 413
- Snails, aquatic palm-leaf traps, 221
- Spurchea praece* in Palestine 409
- Spreue, aetiological and prognostic features, 303
- Sulphamethazine and *E. tenella*, 11
- Tadomus hyattianus* pupation of, 310
- Taenia serialis* optical properties of hooks in polarized light, 322
tanaisiiformis in liver of *u*, 322.
optical properties of hooks in polarized light, 322.
- Thromboangitis obliterans in African women, 321
- Trichomonas foetus* agglutinins of 12
- Trombicula autumnalis*, 6.
- Tropical ulcer extradural block in, 267
- Trypanosomes, electrical charge of 627
- Trypanosoma* sp. in East African skink 221
- Typhus scrub 1
entomology of 2
piderm treatment with chloroquine, 163
in Assam and Burma, 171
Weil Felix reaction, 160
- Vitamin B deficiency 412
deficiency Gold Coast, 277
optic atrophy in, 278.
thiamin therapy 278
- Water beetles, injuries produced by 101
- Wemyss, C. M. Obituary 202, 209
- Wemyss, Miss Mildred G. Theobald Smith Medal, 410
- Yawa, campaign in Sierra Leone 247
in Tanganyika African, description of photographs, 221
treatment of, 250.
treated by acetylarsan and bismuth iod with potassium tartrate 22
Kahn and Ide tests, 22
- Yellow fever epidemiology in Central Africa, 311
in Sierra Leone, 222
pamphlet dated 1750 12

